METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ILAPRAZOLE AND DOMPERIDONE IN CAPSULE DOSAGE FORM BY UPLC

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In partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHARMACY
IN
BRANCH-V-> PHARMACEUTICAL ANALYSIS

Submitted By

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Under the Guidance of

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CERTIFICATE

This is to certify that the project entitled,**"METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ILAPRAZOLE AND DOMPERIDONE IN CAPSULE DOSAGE FORM BY UPLC"**submitted **by Regd. No. 261430013**in partial fulfillment for the award of degree of **Master of Pharmacy (Pharmaceutical Analysis).** The project was carried out at IDEAL ANALYTICAL LAB, PONDYCHERRY and C.L.Baid Metha College of Pharmacy, Chennai-97 under my supervision during the academic year 2014-2016.

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To Whom So ever it may concern

We wish to inform you that **Ravi Dasgupta (Reg .No .261430013)** from C.L BAID METHA COLLEGE OF PHARMACY,THORAIPAKKAM,CHENNAI,had done the M.Pharmacy project work**(METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ILAPRAZOLE AND DOMPERIDONE IN CAPSULE DOSAGE FORM BY UPLC)in our institution from 12/10/2015 to 07/09/2016.**

Thanking You,

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DECLARATION

I hereby declare that this dissertation entitled, **"METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ILAPRAZOLE AND DOMPERIDONE IN CAPSULE DOSAGE FORM BY UPLC"**has been originally carried out by me under the guidance and supervision of **Dr. C.N Nalini, M. Pharm., Ph.D.,** Department of Pharmaceutical Analysis, C.L.Baid Metha College of Pharmacy, Chennai – 97 during the academic year 2014-2016. The work embodied in this thesis is original, and is not submitted in part or full for any other degree of this or any other University.

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Acknowledgement

“Success is how high you bounce when you hit bottom”

“If you can dream it, you can do it”

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LIST OF ABBEREVIATIONS

ACN	:	Acetonitrile
API	:	Active Pharmaceutical Ingredient
AR	:	Analytical Reagent
AU	:	Absorbance unit
BP	:	British Pharmacopoeia
DPD	:	Domperidone
FDA	:	Food and Drug Administration
gm	:	Gram
HPLC	:	High-Performance Liquid Chromatography
ICH	:	International Conference on Harmonization
ID	:	Internal diameter
IP	:	Indian Pharmacopoeia
IPZ	:	Ilaprazole
LC	:	Liquid Chromatography
LOD	:	Limit of Detection
LOQ	:	Limit of Quantification
mg	:	Milligram
µg	:	Microgram

min	:	Minute
ml	:	millilitre
N	:	Theoretical Plates
NMT	:	Not More Than
NLT	:	Not Less Than
nm	:	Nanometer
QA	:	Quality Assurance
QC	:	Quality Control
R^2	:	Correlation Coefficient
RP-HPLC	:	Reverse Phase High-Performance Liquid Chromatography
RP-UPLC	:	Reverse Phase Ultra-Performance Liquid Chromatography
RS	:	Related Substance
RSD	:	Relative Standard Deviation
S/N	:	Signal / Noise
SD	:	Standard Deviation
UPLC	:	Ultra- Performance Liquid Chromatography
USP	:	United States Pharmacopoeia
v/v	:	Volume / Volume
WHO	:	World Health Organization

INTRODUCTION

1. INTRODUCTION

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Analytical chemistry involves separation, identification, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry that derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis is required before a quantitative analysis can be undertaken.

Analytical chemistryis defined asthescience and art of developing sensitive, reliable and accurate method for determining the composition of materials in terms of elements or compounds which they contain. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of drug and chemicals Pharmaceutical analysis deals mainly with bulk materials, dosage forms and more recently, biological samples in support of bio-pharmaceutical and pharmacokinetic studies.[1]

Pharmaceutical analysts in research and development (R&D) of pharmaceutical industry plays a very comprehensive role in new drug development and follow up activities toassure that a new drug product meets the established standards, its stability and continued to meet the purported quality throughout its shelf life.

The different activity of R&D includes drug development, (synthesis and manufacture), formulation, clinical trials, evaluations and finally launching i.e. finished products. Closelyassociated with these are regulatory and quality assurance functions.

Analytical method development and validation is a good research in the field of Pharmaceutical analysis, utilized to determine the drug content in bulk and pharmaceutical dosage forms and in biological fluids like blood, serum, urine etc. In view of the industrial scenario and literature, it was noted that chromatographic techniques like HPLC, LC–MS/MS methods have

created revolutionary precision and accuracy in quantification of drugs in Formulation and in biological fluids even at low concentration.[2]

Need for pharmaceutical Analysis[3]

- ❖ New drug development.
- ❖ Method Validation as for ICH Guidelines
- ❖ Research in Pharmaceutical Sciences
- ❖ Clinical Pharmacokinetic Studies

When promising results are obtained from explorative validation performed during the method development phase, then only full validation should be stared. The process of validating a method cannot be separated from the actual development of method conditions.

A diversity of analytical techniques such as spectroscopy (UV-Visible), gas chromatography (GC), high performance liquid chromatography (HPLC), Ultra performance liquid chromatography (UPLC) supercritical fluid chromatography (SFC), capillary electrophoresis (CE) coupled with selective detectors (diode-array detector (DAD) and mass spectrometry (MS) are normally used to accomplish the above requirements.

In spite of various techniques existing, HPLC and UPLC has become a universal tool for pharmaceutical and biomedical research, as well as product analysis. The accessibility of fully automated systems, excellent quantitative precision, accuracy, sensitivity, selectivity and increased selection of column stationary phases, applicability to a lane variety of sample, Matrices and ability to hyphenate with several spectroscopic detectors has made HPLC or UPLC the instrument of choice for the analysis of most categories of drugs.

Correspondingly, HPLC or UPLC methods are profusely used in the field of biomedical analysis, viz. therapeutic drug monitoring, pharmacokinetic and bioequivalence studies. The assay of drugs in blood, plasma and tissues presents analytical challenges. The drug substance is typically present at low concentrations, bound to proteinaceous material and endogenous compounds typically present in the samples can interfere with the analysis. For these reasons, the analytical methods usually be highly sensitive to detect analytes at low concentrations and required a sample pre-treatment procedure such as liquid-liquid extraction (LLE) or solid phase extraction (SPE), to isolate the analyte from the complex biological matrix. Hence, high sensitivity and automation of sample processing tools to deal with large number of samples are strong incentives for the consideration of HPLC or UPLC methods in biomedical analysis.

UPLC comes from HPLC .Ultra Performance Liquid Chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption. This enhanced the demand for UPLC methods for the simultaneous determination of drugs in pharmaceutical mixtures.[4]

1.1 TYPES

Traditionally, analytical chemistry has been split in to two main types, qualitative and quantitative:

1.1.1 Qualitative

- Qualitative inorganic analysis seeks to establish the presence of a given element or inorganic compound in sample.
- Qualitative organic analysisseeks to establish the presence of a givenfunctional group or Organic compound in a sample.

1.1.2Quantitative

- Quantitative analysis seeks to establish the amount of given element or compound in a sample.

Most modern analytical chemistry is categorized by two different approaches such asAnalyticaltargets or analytical methods[5].

- **By Analytical Targets**
- Bio analytical chemistry
- Material analysis
- Chemical analysis
- Environmental analysis
- Forensics

- **By Analytical Methods**
- Spectroscopy
- Mass spectroscopy
- Spectrophotometry and colorimetric
- Chromatography and Electrophoresis
- Crystallography
- Microscopy
- Electrochemistry

- **Traditional analytical techniques**
- Titration
- Gravimetry
- Inorganic qualitative analysis

- **Instrumental analysis**
- Spectroscopy
- Mass spectroscopy
- Crystallography
- Electrochemical Analysis
- Thermal analysis
- Separation
- Hybrid Techniques
- Microscopy

Analytical chemistry research is largely driven by performance (sensitivity, selectivity, robustness, linear range , accuracy, precision, and speed), and cost (purchase, operation, training, time, and space).

Analytical chemistry has played critical roles In the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing, forensic science and so on.

Pharmaceutical analysis plays a very significant role in quality control of pharmaceuticals through a rigid check on raw materials used in manufacturing of formulations and on finished products. It also plays an important role building up the quality productsthrough

in processquality control. Pharmaceutical analysis is the application of principles of analytical chemistry to drug analysis.

The analytical chemistry may be defined as the science of developing sensitive, relative and accurate methods for determining the composition of materials in terms of elements or compounds which they contain.The most importantcomponent aspectanalysis is quantitative chemical analysis. In the presence age, the physical, chemical andbiological analysis, involve computerized techniques to facilitate better result.[6]

1.2. ANALYTICAL TECHNIQUES[7]

SPECTROSCOPY	APPLICATIONS
Atomic Absorption and Emission spectroscopy(AAS/AES)	To analyse alkali and alkaline earth metals indilute solution, natural liquids, and extracts at trace levels.
Ultraviolet-visibleSpectroscopy (UV/Vis)	To analyse molecular (organic) and ionic species capable ofabsorbingat UV orVisible wave lengths in dilute solutions.
Fourier Transform infraredSpectroscopy(FT-IR)	To analyse only molecular compounds(Organiccompounds, natural products, polymers, etc.).
Fourier Transform RamanSpectroscopy (FT-Raman)	To analyse molecular (organic) compounds which are not responding well in the IR region and hence, it is an alternative IR.
Nuclear Magnetic ResonanceSpectroscopy	To identify and characterize the organic and inorganic compounds.
Microwave spectroscopy	To analyse simple gaseous molecules in far in region to study their stereo chemistry.
Electron spin resonance Spectroscopy (ESR)	To study the information and lifetime of the free radicals formed in organic reactions and all finds applications in biological works.
Molecular Fluorescencespectroscopy	To study the molecular and ionic compounds in dilute solutions capable giving fluorescence, applications in vitamin analysis.

1.2.1 CHROMATOGRAPHY

High Performance Liquid chromatography	To separate and analyse complex mixtures or solutions which include liquids and solids and solids of both organic and inorganic origins.
Gas chromatography	To separate and analyse mixtures of volatile organic compounds, solvent extracts and gases.

1.2.2 THERMAL ANALYSIS

Thermo gravimetric analysis (TGA)	To study the mass charges of materials like polymers, glasses, ceramics, etc., such as evaporation ,decomposition gas absorption, desorption, dehydration etc.,
Thermo mechanical analysis (TMA)	To study the expansion coefficient of composite and laminate materials.
Differential thermal analysis (DTA)	To study the exothermic and endothermic behaviour of clay materials, ceramics, ores, etc.,

1.2.3 X-RAY TECHNIQUE

X-ray fluorescence(XRF) Spectrometry	To identify the elements and their states present in the surface of the materials.
X-ray Diffractometry(XRD)	To study the crystalline properties of solid substances.

1.2.4 MICROSCOPY

Scanning electron microscopy(SEM)	To Study the topography, electronic structure and compositions of metals, ceramics, polymers, composites and biological materials.
Transmission electron microscopy(TEM)	To study the local structures, morphology, and Dispersion of multi-component polymers, cross sections of crystallizations of metallic alloys, semiconductors, microstructure of composites, etc.
Scanning probe microscopy(SPM)	To study the hardness and topography of materials like ceramics, polymers, composites, etc. on a nano scale range.

1.2.5 ELECTRO-CHEMICAL TECHNIQUES

Polarography	To study and determine metals, metal complexes, and organic compounds in trace levels.
Capillary electrophoresis(CE)	To study and characterize biologically active compounds like proteins, amino acids and bio- molecules.

1.2.6 MISCELLANEOUS TECHNIQUES

Total organic carbon analyser (TOC)	To monitor pollutants in environmental studies by determining the carbon contents of the trace compounds.
Elemental Analyser(CHN/S)	To estimate percentage compositions of elements like carbon, hydrogen, nitrogen, and sulphur present in newly synthesized organic compounds, pharmaceuticals, etc.
Polarimetry	To analyse and quantitative optically active compounds like sugar.
Circular Dichroism(CD) and optical Rotatory Dispersion(ORD)	To get the structural information of optically active compounds like, amino acids, proteins etc.
Vibrational Circular Dichroism(VCD) and Vibrational Linear Dichroism(VLD)	Same as above but in the IR region. VLD measurement is employed to study the molecular orientations of thin polymer films.
Mass spectrometry(MS)	To identify the organic compounds. Often used as detectors with HPLC and GC.
Laser Light scattering system(LLIS)	In the study ofmacromolecules like polymers, Gels, proteins, etc. for determining molecular mass and size and their associations.

1.3 CHROMATOGRAPHY

1.3.1 Introduction to Chromatography

Chromatography is relatively a new technique which was first invented by M. Tswett, a botanist in 1906 in Warsaw. In that year, he was successful in doing the separation of chlorophyll, Xanthophyll and several other coloured substances by percolating vegetable extracts through a column of calcium carbonate. The calcium carbonate column acted as an adsorbent and the different substances got adsorbed to different extent and this gives rise to coloured bands at different positions, ion the column. Tswett termed this system of coloured bands as the chromatogram and the method as chromatography after the Greek words Chroma and graphs meaning "colour" and "writing" respectively. However, in the majority of chromatographic procedures no coloured products are formed and the term is a misnomer.

Chromatography is a non-destructive procedure[8] for resolving a multi-component mixture of trace, minor, or major constituents into its individual fractions. Different variations may be applied to solids, liquids, and gases. While chromatography may be applied both quantitatively, it is primarily a separation tools.

1.3.2 Definition of Chromatograph

Chromatography may be defined as a method of separating a mixture of components into individualcomponents through equilibrium distribution between two phases. Essentially, the technique of chromatography is based on the differences in the rate at which the components of a mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called moving phase)[8].

The chromatography method of separation [8], in general, involves the following steps:

1. Adsorption or retention of a substance or separation, in general involves the following steps:
2. Separation of the adsorbed substances by the mobile phase.
3. Recovery of the separated substances by a continuous flow of the mobile phase, the method being called elution.
4. Qualitative and quantities analysis of the eluted substances.

1.3.3 Classification of Chromatographic Techniques

- **According to nature of stationary and mobile phase:**
  - Gas Solid chromatography
  - Gas liquid chromatography
  - Solid liquid chromatography
  - Liquid liquid chromatography
- **According to mechanisms of separation:**
  - Adsorption chromatography
  - Partition chromatography
  - Ion exchange chromatography
  - Molecular exclusion chromatography
  - Affinity chromatography
  - Chiral chromatography

1.3.3.1 Adsorption Chromatography:

Adsorption chromatography[8]is probably one of the oldest types of chromatography around. It utilizes a mobile liquid or gaseous phase that is adsorbed onto the surface of a stationary solid phase. The equilibration between the mobile and stationary phaseaccounts for the separation of different solutes.

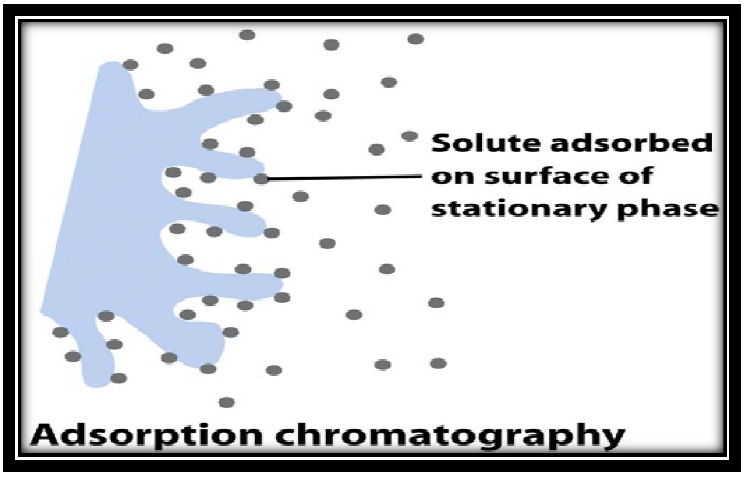


Figure 1.1 Adsorption phenomenon in chromatography

1.3.3.2 Partition Chromatography:

This form of chromatography is based on a thin film formed on the surface of a solidsupport by a liquid stationary phase. Solute equilibrates between the mobile phase and thestationary liquid.

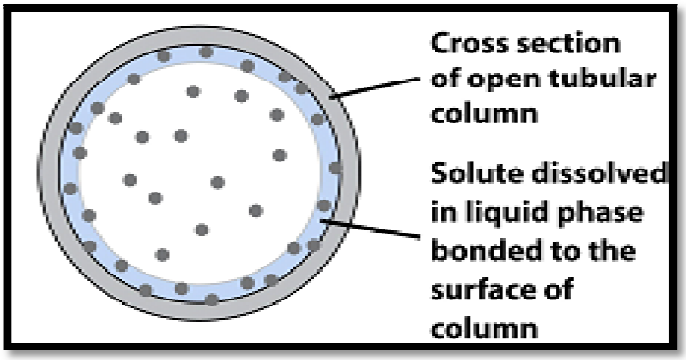


Figure 1.2Partition phenomenon in chromatography

1.3.3.3 Ion Exchange Chromatography:

In this type of chromatography, the use of a resin (the stationary solid phase) is used to covalently attach anions or captions onto it. Solute ions of the opposite charge in themobile liquid phase are attracted to the resin by electrostatic forces.

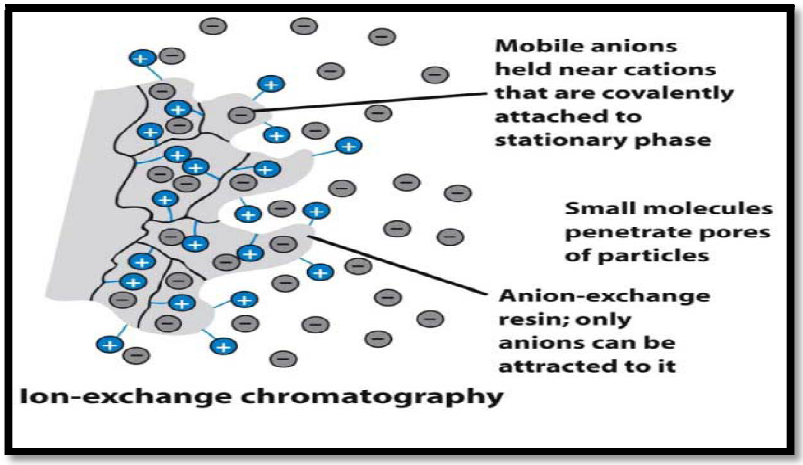


Figure 1.3Ion exchange phenomenon in chromatography

1.3.3.4 Molecular Exclusion Chromatography:

Also known as gel permeation or gel filtration[8], this type of chromatography lacks an Attractive interaction between the stationary phase and solute. The liquid or gaseous Phase passes through a porous gel which separates the molecules according to its size.

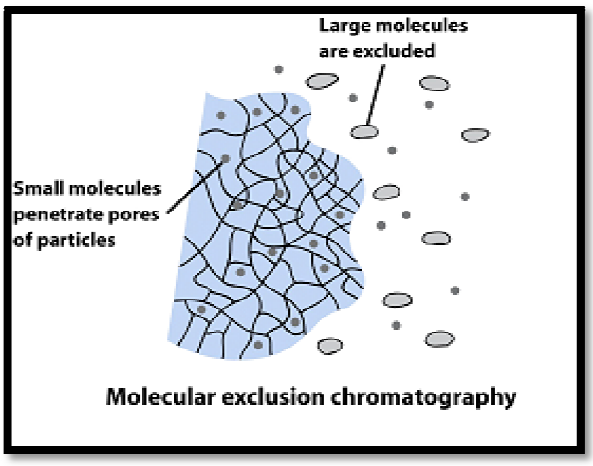


Figure 1.4Molecular Exchange phenomenon in chromatography

The pores are normally small and exclude the larger solute molecules, but allow smaller molecules to enter the gel, causing them to flow through a larger volume. This causes thelarger molecules to pass through the column at a faster rate than the smaller ones.

1.3.3.5 Affinity Chromatography:

Affinity chromatography[8] uses the affinity of the sample with specific stationary phases. This technique is mostly used the field of biotechnology, micro biology, biochemistry etc.

1.3.3.6 Chiral Phase Chromatography:

Separations of opticalisomer can be done by using stationary phases different principles operate for different types of stationary phasesand different samples.The stationary phases are used for this type ofchromatography aremostly chemically bonded silica gel[8]. In chromatographic separation, HPLC and HPTLC methods have widely been exploited in pharmaceutical analysis because of itssimplicity, precision, accuracy, andreproducibility of results.

1.3.3.7 High Performance Liquid Chromatography

The acronym HPLC, coined by the late Prf. Casaba Horvath for his 1970 Pittston paper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns[9].

In the beginning, pumps only had a pressure capability of 500 psi. This was called high pressure liquid chromatography, or HPLC.

The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi of pressure, and incorporated improved injectors, detectors, and columns. HPLC really began to take hold in the mid-to late-1970s[9].

With continued advances in performance during this time (smaller particles, even higher pressure), the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High performance liquid chromatography[9] is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid.

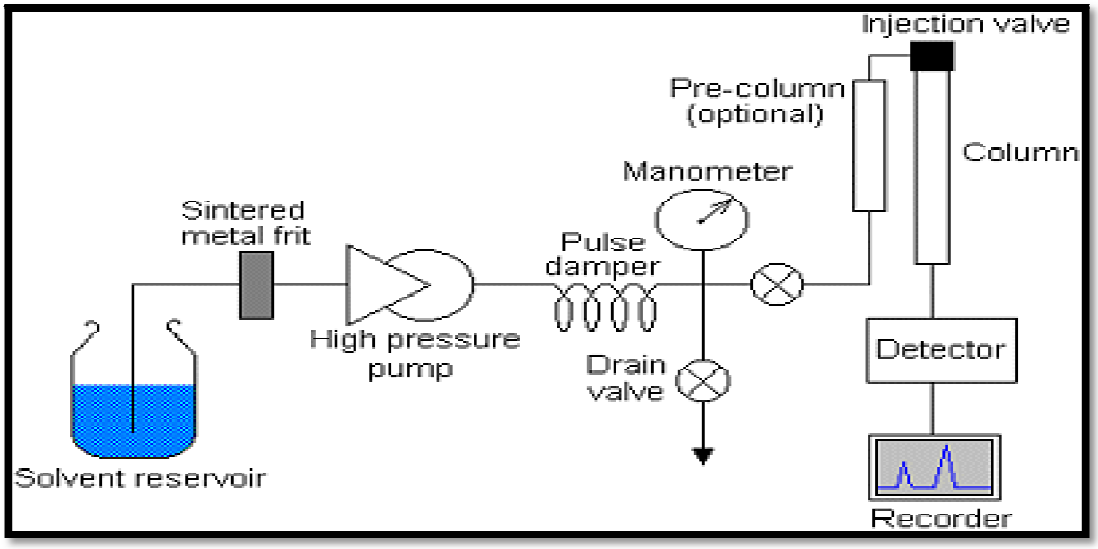


Figure 1.5High-Performance Liquid Chromatography [HPLC] System

Today, compounds in trace concentrations as low as parts per trillion may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, neutraceuticals, cosmetics, environmental matrices, forensic samples and industrial chemicals.

The components of a basic high-performance liquid chromatography (HPLC) system are shown in the simple diagram in Figure 1.5.

A reservoir (Solvent Delivery) holds the solvent (called the mobile phase, because it moves). A high-pressure pump solvent manager is used to generate and meter a specified flow rate of mobile phase, typically millilitres per minute.

An injector (sample manager or auto sampler) is able to introduce (inject) the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column.

The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column (most compounds have no colour, so we cannot see them with our eyes).

The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the elute containing that purified compound for further study. This is called preparative chromatography. The high-pressure tubing and fittings are used to interconnect the pump, injector, column, and detector components to form the conduit for the mobile phase, sample, and separated compound bands.

The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitative the concentration of the sample constituents. Since sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb ultraviolet light, a UV-absorbance detector is used. If the compound fluoresces, a fluorescence detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an evaporative-light-scattering detector (ELSD). The most powerful approach is the use multiple detectors in series.

For example, a UV and/or ELSD detector may be used in combination with a mass spectrometer (MS) to analyse the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.

1.3.3.8 Ultra Performance Liquid Chromatography

In 2004, further advances in instrumentation and column technology were made to achieve very significant increases in resolution, speed, and sensitivity in liquid chromatography. Columns with smaller particles (1.7 micron) and instrumentation with specialized capabilities designedto deliver mobile phase at 15,000 psi (1,000 bars) were needed to achieve a new levelof performance. A new system had to be holistically created to perform ultra-performance liquid chromatography[10], now known as UPLC technology**.**

Basic research is being conducted today by scientists working with columns containing even smaller than 1-micron-diameter particles and instrumentation capable of performing at100,000 psi.

This provides a glimpse of what we may expect in the future. UPLC refers to Ultra Performance Liquid Chromatography[10]. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption. UPLC is comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation.

Figure 1.6Ultra-Performance Liquid Chromatography [UPLC] Instrument

An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates according to the common Van Demeter equation. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance**.**

The classic separation method of HPLC (High Performance Liquid Chromatography)[10] has many advantages like robustness, ease of use, good selectivity and adjustable sensitivity. The main limitation is lack of efficiency compared to gas chromatography or the capillary electrophoresis due to low diffusion coefficients in liquid phase, involving slow diffusion of analytes in the stationary phase. The Van Demeter equation shows that efficiency increases with the use of smaller size particles but this leads to a rapid increase in back pressure, since most of the HPLC system can operate only up to 400 bar. That is why short columns filled with particles of about 2µm are used with these systems, to accelerate the analysis without loss of efficiency.

To improve the efficiency of HPLC separations, the following can be done,

(1) Work at higher temperatures

(2) Use of monolithic columns

1.3.3.8.1Principle

The UPLC is based on the principal of use of stationary phase consisting of particles less than2 µm (while HPLC columns are typically filled with particles of 3 to 5 µm). The underlying principles of this evolution are governed by the van Demeter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).

The Van Demeter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with small diameter particles is much greater than for larger diameters.

$$H=A+B/v+Cv$$

Where;

A, *B*and *C*are constants

Vis the linear velocity, the carrier gas flow rate.

- The *A* term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform.
- The *B* term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by *v*.
- The *C* term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus this term is proportional to *v*.

Therefore it is possible to increase throughput, and thus the speed of analysis without affecting the chromatographic performance. The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistentwith the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency is proportional to column length and inversely proportional to the particle size. Therefore, the column can be shortened by the same factor as the particle size without loss of resolution. The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality.

- **Advantages**
  - Drastically decreases the run time compared to HPLC.
  - Provides selectivity, sensitivity and dynamic range of LC analysis.
  - The time spent on optimizing new methods can also be greatly reduced.
  - Expands the scope of Multi residue method
  - UPLC’s fast resolving power quickly quantifies related and unrelated compounds.
  - Use of very fine particle size of novel separation materials reduces the analysis time.
  - Operation cost is reduced and less solvent consumption.
  - Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches, or the need to re-work material.
  - The time needed for column equilibration while using gradient elution and during method validation is much shorter.

➢ **Disadvantages**

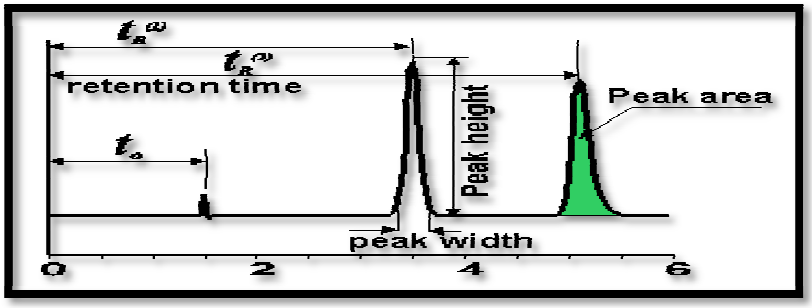
- One of the major disadvantages in UPLC is the higher back pressures compared to conventional HPLC which in turn may reduce the life of the columns.
- This backpressure can be reduced by increasing the column temperature.
- In addition, the phases of less than 2µm are generally non regenerable and thus have limited use.

Table 1.1Comparison between and HPLC and UPLC

Characteristics	HPLC	UPLC
Particle size	3 to 5m	Less than 2m
Maximum back pressure	35-40MP_a	103.Mp_a
Analytical column	Altima C_{18}	Acuity UPLC BEH C_{18}
Column dimensions	150X3.2mm	150X2.1mm
Column temperature	30^0_C	65^0c
Injection volume	5µL(std.In100% Me OH)	2µL(std.In100% ME OH)[7]

1.3.4Parameters in chromatography

❖ **Retention time(R_t)**



- Retention time[11] is the time of elution of peak maximum after injection of compound.

❖ **Retention volume (V_r)**

Retention volume[11] is the volume of mobile phase to elute 50% of the compound from the column. It is the product of retention time and flow rate.

Component Retention volume (V_r) could split into two parts:

- **Reduced retention volume** is the volume of the eluent that passed through the column while the component
- **Dead volume** is the volume of the eluent that passed the column while the component was moving with the liquid phase.

❖ **Separation factor(S):**

Separation factor[11] is the ratio of partition coefficient of the two components to be separated.

It can be expressed and determined by using the following equation:

$$S=\mathbf{Kb}/\mathbf{Ka}=t_{b-t0}/t_a-t_0$$

Wheret_0 = retention time of unretained substance.

Kb = partition coefficients of b and a

t_b,ta = retention time of substance of band a

S = depends in liquid phase, column temperature

If there is more difference in partition coefficient between two compounds, the peaks are far apart and the separation factors are more. If the separation coefficients of two compounds are similar, then the peaks are closer and the separation factor is less.

❖ **Capacity factor (k')**

It is the measure of how well the sample molecule is retained by the column during an isocratic separation. It is affected by solvent composition, separation and aging and temperature of separation[11].

$$K' = V_1-V_0/V_0$$

Where V_1 = retention volume at apex of the peak.

V_0 = void volume of system where an unretained component elutes.

❖ **Selectivity (α)**

- The selectivity is a measure of relative retention of two components in a mixture.
- Selectivity[11] is the ratio of the capacity factors of both corresponding peaks.
- It can be calculated by following formula:

$$\alpha = \frac{t_2 - t_1}{t_1 - t_0}$$

❖ **Resolution (r):**

Resolution expresses the separation of two components in a mixture that determined by the following equation:

$$R = \frac{2(t_2 - t_1)}{W_2 + W_1}$$

Where,

❖ $t_2$and t_1 are the retention times of the two components.

❖ $w_2$andw_1 are the corresponding widths at the bases of the peaks obtained by

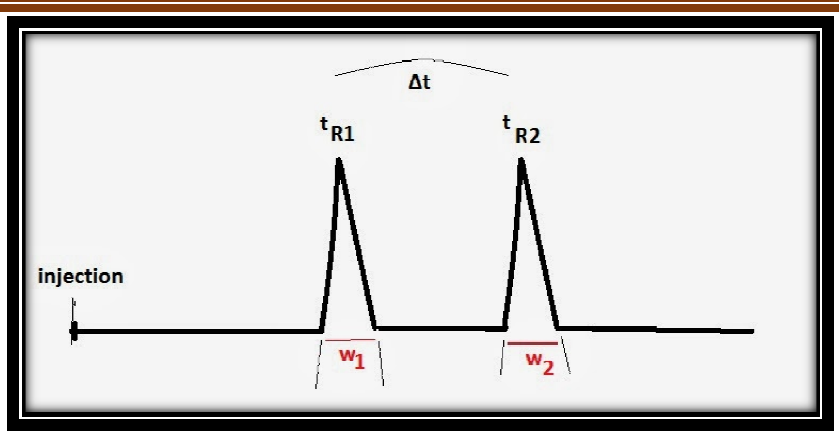
❖ Extrapolating the relatively straight sides of the peaks to the baseline.

Where electronic integrators are used, it may be convenient to determinethe resolution, R, by the following equation:

$$R = \frac{2\,(t_2 - t_1)}{1.70\,(W_{2h/2} + W_{1h/2})}$$

Where by W1 h/2 andW2 h/2 are the widths at half-height of corresponding peaks.

For better separation, the ideal value of R is 1.5



❖ **Theoretical plates (N):**

The number of theoretical plates[11] is a measure of column efficiency. It is expressed by following equation:

$$N = 16\left(\frac{t}{W}\right)^2 = 5.54\left(\frac{t}{W_{h/2}}\right)^2$$

Where,

- T is retention time of the peak
- W is the width of the peak.
- $W_h/2$ is width at half-height of the peak.

- Value of theoretical plates, higher than 4000 is indicating good column performance.

❖ **Height Equivalent to a Theoretical Plate(HETP)**:

A theoretical plate[11] is an imaginary or hypothetical unit of a column where distribution of solute between stationary phase and mobile phase has attained equilibrium. It can also be called as a functional unit of the column.

A theoretical plate can be of any height, which describes the efficiency of separation. If HETP is less, the column is more efficient. If HETP is more, the column is less efficient.

HETP =length of the column /no. of theoretical plates

HETP is given by Van Demeter equation**.**

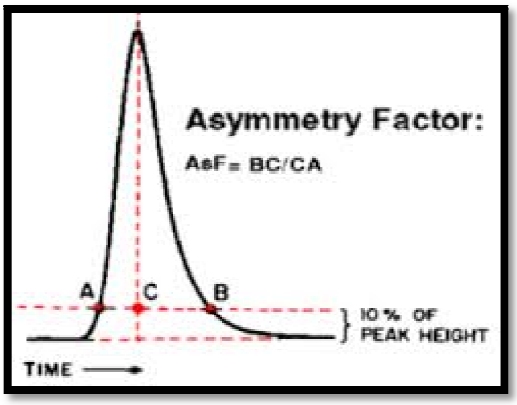
$$HETP = A+B/u+Cu$$

Where

A=Eddy diffusion term or multiple path diffusion which arises due to the packing of the column. This can be minimized by uniformity of packing.

B	=	longitudinal diffusion term or molecular diffusion.
C	=	Effect of mass transfer.
U	=	flow rate or velocity of mobile phase.

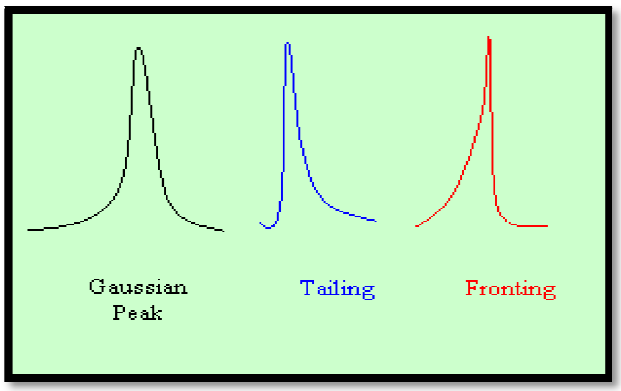
❖ **Asymmetry factor**:



The asymmetry factor is a measure of peak tailing. It is defined as the distance from the center line of the peak to the back slope divided by the distance from the center line of the peak to the front slope, with all measurements made at 10% of the maximum peak height.

- **Fronting:**

Fronting[11] is due to the saturation of stationary phase and can be avoided by using less quantity of sample.



- **Tailing:**

It is due to the more active adsorption sites can be eliminated by support pretreatment, more polar mobile phased increasing the amount of liquid phase.

1.3.5 Method Development and Optimization of Chromatographic Conditions

Methods for analysing drugs in multi component dosage forms can be developed if the nature of the sample, namely, its molecular weight, polarity, ionic character and the solubility parameter are in hand. Until and unless considerable trial and error procedures have not performed, an exact recipe for HPLC cannot be provided[12].

The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase. In general one begins with reversed phase chromatography, when the compounds are hydrophilic in nature with many polar groups and are water soluble**.**

1.3.5.1Selection of stationary phase/ column:

Selection of the column is the initial and the most noteworthy step in method development.

The proper choice of separation column includes three different approaches.

1. Selection of separation system
2. The particle size and the nature of the column packing
3. The physical parameters of the column i.e. the length and the diameter.

Some of the crucial parameters considered while selecting chromatographic Columns. They are length and diameter of the column, packing material, Shape of the particle, Size of the particles, % of Carbon loading, Pore volume, Surface area and End capping. The column is selected depending on the nature of the solute and the information about the analyte, Reversed phase mode of chromatography facilitates information about the analyte.

Reversed phase mode of chromatography[12] facilitates a wide range of columns like dim ethylsilane (C2), butylsilane (C4), octylsilane (C8), octadecylsilane (C18), base deactivated silane (C18) BDS, cyanopropyl (CN), nitro, amino etc.

1.3.5.2Selection of mobile phase:

The primary objective in selection and optimization of mobile phase[12] is to achieve optimum separation of all the individual impurities and degrades from one other and from analyte peak. In liquid chromatography, the solute retention is governed by the solute distribution factor, which reflects the different interactions of the solute-stationary phase, solute-mobile phase and the mobile phase-stationary phase.

For a given stationary phase, the retention of the given solute depends directly upon the mobile phase, the nature and the composition of which has to be judiciously selected in order to get appropriate and required solute retention. The mobile phase has to be adapted in terms of elution strength (solute retention) and solvent selectivity (solute separation).

Solvent polarity is the key word in chromatographic separations since a polar mobile phase will give rise to low solute retention in normal phase and high solute retention in reverse phase LC.

The selectivity will be particularly altered if the buffer P^H is close to thepKaof the analytes; the solvent strength is a measure of its ability to pull analyte from the column. It is generally controlled by the concentration of the solvent with the highest strength. Buffers, pH of the buffer, mobile phase composition are the parameters, which shall be taken into consideration while selecting and optimizing the mobile phase.

Firstly, Buffer and its strength play an important role in deciding the peak symmetries and separations. Some of the most, commonly employed buffers are Phosphate buffer (Potassium di hydrogen phosphate, Di-potassium hydrogen phosphate, Sodium dihydrogen phosphate, Disodium hydrogen phosphate), Phosphoric acid buffers prepared using O-Phosphoric acid, Acetate buffers (Ammonium acetate, Sodium acetate) and Acetic acid buffers prepared using acetic acid.

The retention time[12] also depends on the molar strengths of the buffer. Molar strength is increasingly proportional to retention times. The strength of the buffer can be increased, if necessary, to achieve the required separations. The solvent strength is a measure of its ability to pull analytes from the column. It is generally controlled by the concentration of the solvent with the highest strength. Secondly, pH plays an important role in achieving the chromatographic separations as it controls the elution properties by controlling the ionization characteristics.

Ware conducted using buffers having different pH to obtain the required separations. It is important to maintain the pH of the mobile phase in the range of 2.0to8.0 as most columns do not

withstand the pH which is outside this range. This is due to the fact that the Siloxane linkage area gets cleaved below pH 2.0, while pH valued is above 8.0, silica may dissolve.

Finally, by choosing the optimum mobile phase[12] composition most of the chromatographic separations can be achieved. This is due to the fact that fairly large amount of selectivity can be achieved by choosing the qualitative and quantitative composition of aqueous and organic portions. Most widely used solvents in reverse phase chromatography are methanol and acetonitrile. Experiments were conducted with mobile phases having buffers with different pH and different organic phases to check for the best separations between the impurities. A mobile phase which gives separation of all the impurities and degrades from the analyte peak and which is rugged with variations of both aqueous and organic phase by at least ±0.2% is preferred.

The low solubility of the sample in the mobile phase can also cause bad peak shapes. It is always advisable to use the same solvents for the preparation of sample solution for the preparation of sample solution as the mobile phase to avoid precipitation of the compounds in the column injector.

Optimizations are often started only after a reasonable chromatogram has been obtained. A reasonable chromatogram means symmetrical peaks in the chromatogram and detection of all the compounds. By slight modification of the mobile phase composition, the position of the peaks can be predicted within the range of investigated changes. An optimized chromatogram is the one in which all the peaks are symmetrical and are well separated in less run time.

1.3.6Validation

The word "validation"[13] means "Assessment" of validity or action of validity or action of providing effectiveness'.

1.3.6.1 Definitions

FDA defines validation as "establish the documented evidence which provides a high of assurance that a specific process will consistently produce a product of predetermined specifications and quantity attributes"[14].

WHO action of providing that, any procedure, process, equipment, material, activity, or system actually leads to the expected results[15].

EUMGP define validation as "action of proving in accordance with the principle of Good manufacturing practice (GMP), that any material, activity or system actually lead to expected result"[15].

AUSTRALIANGMP defines validation as "the action of proving that any material, process, activity, procedure, system, equipment or mechanism and intended results"[15].

1.3.6.2 Method Validation Guidelines

Regulatory agencies and European community, Japan and United States published a number of guidelines to assist pharmaceutical companies in validation of analytical methods for drug substances[13-15].

Regulatory agencies such as:

- ✓ International Conference on Harmonization (ICH)
- ✓ Food and Drug Administration (FDA)
- ✓ United States Pharmacopoeia (USP)

Table 1.2Literature from industrial committees and regulatory agencies

COMMITTEES AND REGULATORY AGENCIES	GUIDELINES AVAILABLE
ICH	a) $Q2R_1$guidelines are guidelines for new method development and its validation. b) Q1R1guidelines are for development and validation of stability indicating analytical methods includes methodology.
USFDA	Two industry guidelines : a) For the validation of analytical methods. b) For the validation of bio analytical methods
IUPAC	"Harmonized guideline for single laboratory validation of methods of analysis.
EURACHEM	Detailed guide for method validation primarily developed for ISO/IEC accredited. Laboratories but because of its completeness it is also a good source For (bio) pharmaceutical laboratories.
AOAC	Technical document for the verification of analytical methods for the ISO 17025 accreditation.
Huber	Has published a technical document for the verification of analytical methods for the ISO 17025 accreditation.

Table 1.3Comparison of Validation Parameters Required for HPLC Assay Methods

ICH GUIDELINES	USP GUIDELINES	FDA GUIDELINES
Accuracy	Accuracy	Accuracy
Precision	Precision	Precision
Repeatability	--	Repeatability
Inter-day precision	--	Inter-day precision
Reproducibility	--	Reproducibility
Specificity	Specificity	Specificity
Limit of detection	Limit of detection	Limit of detection
Limit of quantification	Limit of quantification	Limit of quantification
Linearity	Linearity	Linearity
Range	Range	Range
--	Ruggedness	--
Robustness	Robustness	Robustness
System suitability	System suitability	System suitability

1.3.6.3 Validation Parameters

- **Accuracy**

The accuracy[14] of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Accuracy can also be described as the extent to which test results generated by the method and the true value agree.

The ICH document on validation methodology recommends accuracy to be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (for example, three concentrations with three replicates each). Accuracy should be reported as percent recovery by the assay of known added amount of standard in the sample or as the difference between the mean and the accepted true value, together with the confidence Intervals.

Thus, accuracy of the method was studied by recovery experiments using standard addition method at three different levels (80%, 100% and 120. Known amounts of Standard solutions containing analyte were added to pre-quantified sample solutions to get 80,100 and 120 %.These samples were analyzed by injecting the sample solution and % recovery was calculated. In the present study %recovery was calculated by the following formula.

$$\frac{\text{AREA OF SPIKED SAMPLE-AREA OF UNSPIKED SAMPLE}}{\text{AREA OF STANDARD}} \times \frac{\text{CONC. OF STANDARD}}{\text{CONC. OF ADDED DRUG}} \times 100$$

Acceptance limit for % recovery is 98-100%

- **Precision:**

The precision[14] of an analytical procedure expresses the closeness of agreement (degree of Scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision is a measure of the reproducibility of the whole analytical method (including sampling, Sample preparation and analysis) under normal operating circumstances. Precision is determined by using the method to assay a sample for a sufficient number of times to obtain statistically Valid results (i.e. between 6 - 1 0). Therefore for present work sample were assayed six times and the %RSD for obtained assay values was obtained. Precision is then expressed as the Relative Standard Deviation.

$$\%RSD = \frac{\text{STD DEV}}{\text{MEAN}} \times 100$$

- **Repeatability:**

Repeatability[14]expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

❖ **Intermediate Precision:**

Intermediate precision[14] expresses within-laboratories variations; different days, different analysts, different equipment, etc. The objective of intermediate precision validation is to verify that in the same laboratory the method will provide the same results once the development phases over. The objective is also extent to verify that the method will provide the same results in different laboratories (ruggedness).

❖ **Reproducibility:**

Reproducibility[14] expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

❖ **Specificity:**

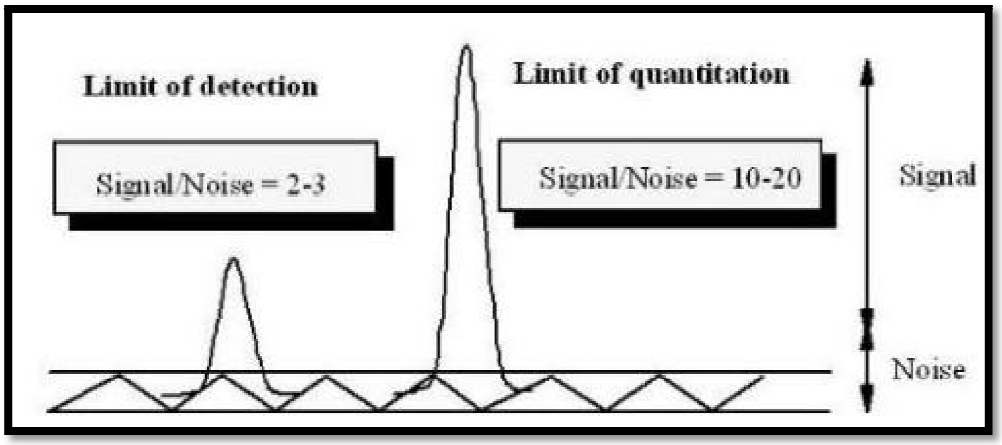
ICH defines specificity as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc."USP<1225>refers to the same definition but also comments that other reputable authorities such as IUPAC and AOAC use the term "selectivity" for the same meaning. This reserves the use of "specific" for those procedures that produce a response for a single analyte only. ISO/IEC most likely has the same understanding because it requires a method to be "Selective" rather than specific. Our goal is to distinguish and quantify the response of the target compounds from the responses of all other compounds Selectivity is the ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix.

Specificity[14] for an assay ensures that the signal measured comes from the substance of interest and that there is no interference from excipient and/or degradation products and/or impurities. Determination of this can be carried out by assessing the peak identity and purity.

❖ **Quantitation:**

- ✓ Limit of detection (LOD) and
- ✓ Limit of quantitation (LOQ) study

Explanation for the LOD and LOQ



❖ **Limit of detection**:

The detection limit[14] of an analytical method is the lowest amount of analytein a sample which can be detected but not necessarily quantitated as an exact value.In chromatography, the detection limit is the injected amount that resultsina peak with a height atleasttwoor three times as high as the baseline noise level. Besides this signal/noise method, LOD can be measured by another three:

Different methods;

(i) Visual inspection

(ii) Standard deviation of the blank response

(iii) Standard deviation of the response based on the slope of the calibration curve.

❖ **Limit of quantitation:**

The quantitation limit[14] of an analytical method is the lowest amount of analyte in a sample which can be quantitated with suitable precision and accuracy. In chromatography, the quantitation limit is the minimum injected amount that produces quantitative measurements in the target matrix with acceptable precision, typically requiring peak heights 10 to 20times higher than the baseline noise. Beside this signal/noise method, LOQ can be measured by another three

Different methods;

(i) Visual inspection

(ii) Standard deviation of the response

(iii) Standard deviation of the response based on the slope of the calibration curve.

❖ **Linearity:**

The linearity[14] of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. This is the method's ability (within a given range) to obtain results which are either directly, or after mathematical transformation proportional to the concentration of the analyte within a given range.

Linearity is determined by calculating the regression line using a mathematical treatment of the results (i.e. least mean squares) vs. analyte concentration. Calibration curve was constructed by plotting peak area versus concentrations of analyte.

❖ **Range:**

The range[14] of an analytical procedure is the interval between the upper and lower concentration (Amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range is normally expressed in the same units as the test results. For assay tests, ICH requires the minimum specified range to be 80 to 120 percent of the test concentration.

❖ **Robustness:**

The robustness[14] of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Thus Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, laboratories, instruments, reagents, assay temperatures, small variations in mobile phase, different days etc. (i.e. from laboratory to laboratory, from analyst to analyst.)

DRUG PROFILE

2. DRUG PROFILE

2.1 ILAPRAZOLE

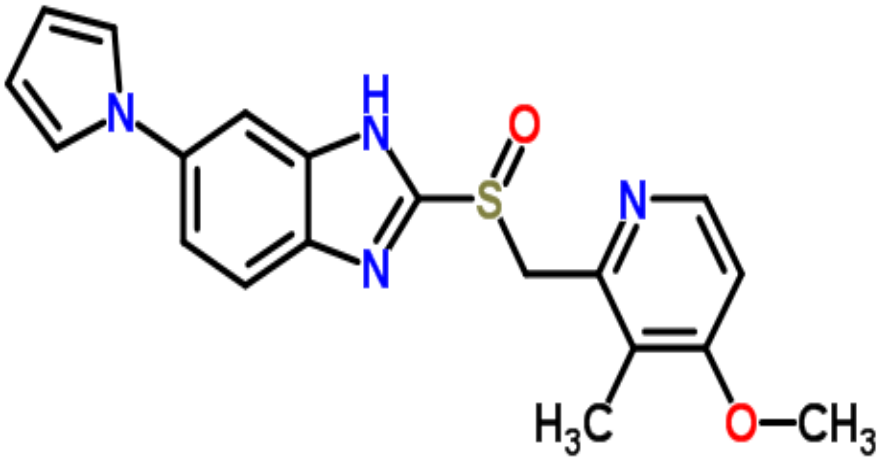
Ilaprazole[16] is a proton pump inhibitor (PPI) used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease (GORD/GERD) and duodenal ulcer. It is available in strengths of 5, 10, and 20 mg.

CAS registry number (Chemical Abstracts Service): 0172152-36-2

Chemical Name : 2-{[(4-Methoxy-3-methyl-2-pyridinyl)methyl]sulfinyl}-5-(1H-pyrrol-1-yl)-1H-benzimidazole

Molecular Formula : $C_{19}H_{18}N_4O_2S$

Chemical structure :



Molecular weight : 366

Density : 1.39g/cm^3

Category : Antacid

Sub Category :Proton pump inhibitor (PPI)

Appearance : A white to yellow powder

Loss on drying :NMT 0.1%

Sulphated Ash : NMT 0.1%

Storage :Store protected from light

Foreign Names :

- Ilaprazolum (Latin)
- Ilaprazole (French)
- Ilaprazol (German)
- Ilaprazol (Spanish)

Brand Names:

- Noltec
  Ilyang, South Korea
- Yi Li An
  Livzon Pharma Group, China

Mechanism of Action: Suppresses gastric basal and suppresses acid secretion by inhibiting the parietal cell H+/K+ ATP pump.

Adverse reactions :

The most common side effects are:

- Nausea and Vomiting
- Dizziness
- Diarrhea
- Constipation
- Abdominal Pain
- Wind and Bloating
- Skin Rashes
- Headaches

Longer Term Side Effects Include:

- Increase Hip Fracture Risk If Over 50 years Old And On Long Term Treatment
- More Susceptible To Iron Deficiency Anemia
- Increased Risk Of Pneumonia In older People
- Vitamin B12 Deficiency

2.2 DOMPERIDONE

Domperidone[17] belongs to the class of organic compounds known as benzimidazoles. These are organic compounds containing a benzene ring fused to an imidazole ring (five member ring containing a nitrogen atom, 4 carbon atoms, and two double bonds).

This drug is developed by Janssen Pharmaceutical that acts as a peripherally selective antagonist of the dopamineD_2 and D_3 receptors. It is administered orally, rectally, or intravenously. Domperidone is used to relieve nausea and vomiting; to increase the transit of food through the stomach (that is, as a prokinetic agent via increasing gastrointestinal peristalsis); and to promote lactation (breast milk production) by release of prolactin. It is also used in scientific research to study the biological function of dopamine, an important neurotransmitter and hormone, in the body.

Domperidone is available in the form of tablets, orally disintegrating tablets (based on Zydis technology), suspension and suppositories.

CAS registry number (Chemical Abstracts Service): 0057808-66-9

Kingdom **:** Organic compounds

Super class **:**Organo heterocyclic compound

Class **:** Benzimidazoles

Appearance **:**A white or almost white powder

Storage **:**Store protected from light

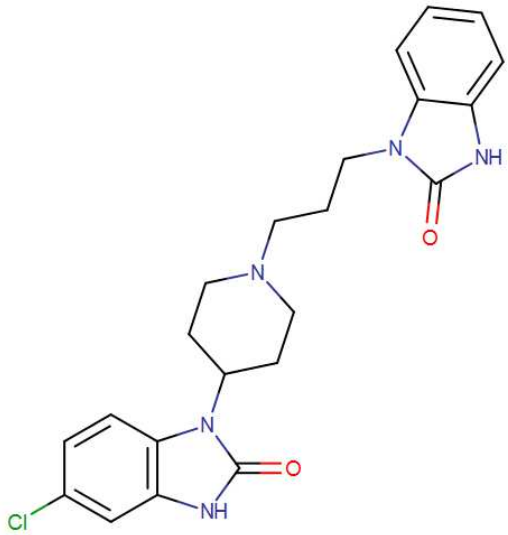
Brand name **:**

- Motilium
- Motaline
- MotinormCosti
- Moperid
- Nomit
- Monlobia
- Brulium
- Motidone
- Molax
- Motilex

Chemical Name **:** 5-chloro-1-{1-[3-(2-oxo-2,3-dihydro-benzoimidazol-1-yl)-propyl]-piperidin-4- yl}-1,3-dihydro-benzoimidazol-2-one

Molecular Formula **:** $C_{22}H_{24}ClN_5O_2$

Chemical structure :



Molecular weight : 425

Solubility :

- Soluble in 0.1M HCl
- Slightly soluble in 0.1M NaOH
- Insoluble in Water

Category : Antiemetic agent, Prokinetic agent

Percentage Purity :≥98%

Loss on drying :NMT 0.5%

Half Life : 7 hours

Protein Binding : 91% - 93%

Foreign Names

- Domperidonum (Latin)
- Domperidon (German)
- Domperidone (French)
- Domperidona(Spanish)

Mechanism of Action: Domperidone is a peripheral dopamine D_2 and D_3 receptor antagonist. It provides relief from nausea by blocking receptors at the chemoreceptor trigger zone (a location in the nervous system that mediates nausea) at the floor of the fourth ventricle (a location near the brain). It increases motility in the upper gastrointestinal tract to a moderate degree and increases lower oesophageal sphincter pressure by blocking dopamine receptors in the gastric antrum and the

duodenum. It blocks dopamine receptors in the anterior pituitary gland increasing release of prolactin which in turn increases lactation. Domperidone may be more useful in some patients and cause harm in others by way of the genetics of the person, such as polymorphisms in the drug transporter gene*ABCB1* (which encodes P-glycoprotein), the voltage-gated potassium channel*KCNH2* gene (hERG/K_v11.1), and the α_{1D}—adrenoceptor*ADRA1D* gene.

Adverse reactions :

- ✓ Penetration of immature blood brain barrier.
- ✓ Cardiac side effects
- ✓ Hyperprolactinemia
- ✓ Mastodynia (breast pain/tenderness)
- ✓ Galactorrhea (inappropriate or excessive milk production/secretion

Uses:

- Antiemetic
- Domperidone is useful in diabetic and idiopathic gastroparesis.
- Domperidone can be used to relieve gastrointestinal symptoms in Parkinson's disease.
- **Functional dyspepsia**: Domperidone may be used infunctional dyspepsia in both adults and children**.**
- **Pediatric reflux:** Domperidone has been found effective in the treatment of pediatricreflux. However some specialists consider it to be an excessively powerful drug for treating babies with reflux.

LITERATURE REVIEW

3. LITERATURE REVIEW

P. Venkateswarlu et al., have reported *"Determination of Ilaprazole and Domperidone in Individual Dosage form Tablets by RP-HPLC"*. A simple, sensitive and precise high performance liquid chromatographic method for the analysis of Ilaprazole and Domperidone was developed, validated and used for the determination of compounds in commercial pharmaceutical products. The compounds were well separated an isocratically on a C18 column inertisil C18, 5 µm, [250 mm × 4.6 mm] utilizing a mobile phase consisting of acetonitrile: phosphate buffer (60:40, v/v, pH 7.0) at a flow rate of 1.0 ml/min with UV detection at 243 nm. The retention time of Ilaprazole and Domperidone was found to be 2.543 min and 7.245 min respectively. The method was found selective and peaks of Ilaprazole and Domperidone were well separated. The proposed method is linear (r2 = 0.999 for Ilaprazole and Domperidone), accurate with 99.5 % recovery for Ilaprazole and 99.4 % recovery for Domperidone and precise (% RSD < 1 %). The method was used to determine potency of commercial product and potency was found within limit.[18]

SnehaJansari K et al.,have reported *"Development and validation of stability indicating method for simultaneous estimation of Ciprofloxacin hydrochloride and Tinidazole using RP-UPLC method"*. The sample was analyzed by reverse phase C18 column (Purospher Star 100×2.1 mm, 2µm) as stationary phase and Phosphate Buffer: Acetonitrile (80:20) as a mobile phase and pH 3.0 was adjusted by Orthophosporic acid at a flow rate of 0.3 ml/min. Quantification was achieved of Ciprofloxacin HCl at 278.5 nm and of Tinidazole at 317.5 nm with PDA detector. The retention time for Ciprofloxacin HCl and Tinidazole was found to be 1.71 and 2.22 minute respectively. The linearity for Ciprofloxacin HCl and Tinidazole was obtained in the concentration range of 3.125-43.75 µg/ml and 3.75-52.5 µg/ml with mean accuracies of 99.77% and 99.75% respectively.[19]

C Rambabu et al.,have reported*"Development and Validation of RP-UPLC Method for Simultaneous Estimation of Abacavir Sulphate and Lamivudine in Combined Tablet Dosage Form"*. Chromatographic separations were carried using Acquity BEH C8 (100 mm x 2.1 mm) 1.7 µm column) with a mobile phase composition of TTriethylamine phosphate buffer (pH 2.5) and methanol in the ratio 50:50% (V/V) have been delivered at a flow rate of 0.5 ml min-1 and the detection was carried out using UV detector at wavelength 230 nm. The retention time for

Abacavirsulphate and Lamivudine were 0.83 and 1.62 minute respectively. The correlation coefficient values in linearity were found to be 0.9999 for both at concentration range 2.509-50.190 µg mL-1 and 20.093 - 401.860 µg mL-1 respectively. The recovery results were found in the range from 99.47- 101.08%.[20]

T. Sivakkumar et al., have reported "*Development and validation of a rapid-chemo metrics assisted RP-HPLC method with PDA detection for the simultaneous estimation of Domperidone and Ilaprazole in pure and pharmaceutical formulation*". A simple, rapid, accurate and precise chemo metrics assisted RP-HPLC method was developed, optimized and validated for the simultaneous estimation of the Domperidone and Ilaprazole in pure and pharmaceutical formulation. The developed method was optimized by using Central composite design (CCD) in resoponse surface methodology (RSM). Separation of Domperidone and Ilaprazole was achieved on phenomenex C18 column (150 X4.6 mm i.d, 5µ particle size) with mobile phase containing 64.89% of acetonitrile and 35.11 % of ammonium acetate buffer (14.18mM) was delivered at a flow rate of 1.2 ml/min. Detection was carried out at 286nm by photodiode array detection. Retention time was found to be 1.661 for Domperidone and 2.420 for Ilaprazole. The developed and optimized method was validated successfully as ICH guidelines. Linearity of Domperidone and Ilaprazole were found to be 30-90µg/ml and 10-30 µg/ml with their correlation coefficient (R2) of 0.9997 and 0.9995 respectively.LOD and LOQ were found to be 158.12ng/ml and 479.16ng/ml for Domperidone and 101.68 ng/ml and 308.12ng/ml for Ilaprazole. The % RSD value of accuracy and precision study was found to be less than 2 %. Hence this proposed method is useful for the simultaneous estimation of Domperidone and Ilaprazole in pure and pharmaceutical dosage form.[21]

Z. N. Patel et al., have reported "*RP-HPLC Method for Simultaneous Estimation of Levosulpiride and Pantoprazole Sodium in Tablet Dosage Form***".** A simple, economic, selective, precise, and stability-indicating RP-HPLC method for analysis of Levosulpiride and Pantoprazole sodium was developed and validated according to ICH guidelines. The quantification of the drug was carried out using Hypercil BDS C18250mm×4.6mm× 5µm or its equivalent in isocratic mode, with mobile phase comprising of Water: Acetonitrile: Triethylamine (60: 40: 0.25 v/v/v) the flow rate was 1ml/min and the detection was carried at 225 nm. The retention time for Levosulpiride and Pantoprazole sodium was found to be 3.597 and 5.147min respectively. The percentage assay for

Levosulpiride and Pantoprazole sodium was found to be 99.56% and 98.44% respectively. The method was also applied for the determination of Levosulpiride and Pantoprazole sodium in the presence of their degradation products formed under variety of stress conditions. Proposed method was validated for precision, accuracy, linearity range, specificity and robustness.[22]

Umadurai M et al., have reported *"Development and validation of a rapid UPLC Assay method for the simultaneous estimation of Paroxetine and Clonazepam in tablet dosage form"*. Chromatographic separation was performed on Thermofischer scientific Hypercel C18 column (50x 2.1mm,1.8µm) column with mobile phase comprising of mixture of Acetonitrile: Methanol: Potassium di hydrogen orthophosphate buffer (8:52:40)buffer (pH 3, adjusted with Ortho phosphoric acid) at the flow rate 0.5 ml/min. The detection was carried out at 265 nm. The retention times of Paroxetine and Clonazepam were found to be 1.28 and 2.45 mins respectively with a run time of 4 mins, theoretical levels for Paroxetine and Clonazepam were 4144and 5067 respectively, with a resolution of 8.09.[23]

Sevak Manan R et al., have reported *"Development& validation of RP-UPLC method for simultaneous estimation of Ofloxacin and Ornidazole in their combine dosage form including stress study"*. The sample was analyzed by RP-UPLC instrument using reverse phase C18 column (Purospher Star 100×2.1 mm, Merck Specialities) as stationary phase and Phosphate Buffer: Acetonitrile (70:30 v/v) as a mobile phase [where PH of the buffer was adjusted to 2.5 by using Tri ethyl amine (1ml / lit buffer) and Orthophosphoric acid at a flow rate of 0. 4 ml/min. TUV detector was used for the detection at 294 nm. The retention time for Ofloxacin and Ornidazole was found to be 0.648 and 1.158 minute respectively. The linearity for both the drugs was obtained in the concentration range of 2-14 µg/ml and 5-35 µg/ml.[24]

ParamasivamBalan et al., have reported *"Development and validation of stability-indicating RP-UPLC method for simultaneous estimation of Thiocolchicoside and Aceclofenac in combined dosage form"*. The chromatographic separation was carried out by Thermo Scientific UPLC Instrument, Accela 1250 Pump, auto sampler with PDA detector, using column Thermo ScientificHypersil gold C18, (50 x 2.1mm) particle size 1.9µm using 5% ammonium acetate buffer and methanol in the ratio of 40:60, pH was adjusted to 5 with Orthophosphoric acid as mobile phase

at a flow rate of 250 µl/min with the detection at 276nm. The run times of the TCC and ACF were about 0.697 and 1.125 minutes, respectively. The detector response is linear from 4.8 µg/mlto 7.2 µg/ml and 63.8 µg/ml to 96 µg/ml concentrations for TCC and ACF respectively. The detection limit and quantification limit was 0.076µg and 0.23µg for TCC and 0.27µg and 0.71µg for ACF. The percentage of assay of TCC and ACF were about 99.50% and 99.96% respectively.[25]

R.A Tamboliet al., *have reported "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ilaprazole and Domperidone in Pharmaceutical Dosage Form"*. A specific, accurate, precise and reproducible RP-HPLC method was developed and subsequently validated for the simultaneous determination of Ilaprazole and Domperidone in pharmaceutical dosage form. The proposed HPLC method utilizes Hypersil (Thermo scientific) C18 column (250 mm × 4.6 mm id, 5 µm particle size), and mobile phase consisting of methanol:phosphate buffer (40:60) and pH adjusted to 4.0 with 0.1M glacial acetic acid at a flow rate of 1.0 ml/min. Quantitation was achieved with UV detection at 230 nm based on peak area with linear calibration curves at concentration ranges 5-15 µg/ml for Ilaprazole and 15-45 µg/ml for Domperidone. The retention time of Ilaprazole and Domperidone were found to be 3.433 min and 5.860 min respectively. The method was validated in terms of accuracy, precision, linearity, limits of detection, limits of quantitation and robustness. This method has been successively applied to marketed formulation and no interference from the formulation excipients was found.[26]

Pradeep G. Shelke et al.,have reported **"***Validated Stability-indicating assay method for determination of Ilaprazole in bulk drug and tablets by high performance liquid chromatography***".** A validated stability-indicating HPLC method was reported for the determination of Ilaprazole in bulk drug and tablet. The drug was subjected to the various stress conditions as per the ICH guidelines. The degradation behavior of Ilaprazole was studied under hydrolytic, oxidative, photolytic and thermal conditions and was found to be unstable in almost all conditions except under alkaline and photolytic conditions. The separation of drug and its degraded products was carried out on Kinetex C- 18 100A (5µ, 250×4.6 mm) column. The initial mobile phase composition used was Acetonitrile and water in the ratio 50:70v/v for 1 min then changed to 70:30v/v in next 6 min and finally equilibrated back to initial composition in 14min. The method was applied for the determination of Ilaprazole in marketed tablet formulation. The detection was carried at 305 nm using PDA detector with a flow rate of 1.0ml/min and injection volume 20µl. The

validation of developed method was performed for linearity, accuracy, precision, selectivity and specificity and robustness**.**[27]

Darshil B. Shah et al., have reported in a review article "*Analytical method for determination of proton pump inhibitors in bulk and indifferent dosage forms*". Proton pump inhibitor is very effectively used in gastric disorder for reducing acid secretion. They very potent in nature and used only after therapy with histamine-2 (H2) receptor antagonists, known as H2 blockers, have been unsuccessful for symptoms of reflux. PPIs are inactivated by exposure to gastric acid, due to rapid degradation of these drugs in acidic and aqueous media, it is challenging to develop analytical method where in stability of drug is least hampered. This review entitles different methods developed for determination of PPIs like UV-Spectroscopy, liquid Chromatography and LC-MS.[28]

Kamala Govinda Rao et al., have reported "*Method Development and Validation for Simultaneous Estimation of Omeprazole and Domperidone by RP-HPLC*". The developed RP-HPLC method allows rapid and precise determinations of Omeprazole and Domperidone, the scope of the present work was to expand and optimization of the chromatographic conditions, to develop RP-HPLC method. A series of mobile phases were tried, among the various mobile phases as methanol and 0.1% ammonium acetate an ideal mobile phase, since it gave a good resolution and peak shapes with perfect optimization. The flow rate was optimized at 1 ml/min. The linearity and correlation coefficient of Omeprazole and Domperidone were found to be 10–50 ug/ml, and 10.10–50.50 ug/ml 0.9996, and 0.998 respectively. The limit of detection for Omeprazole and Domperidone was found to be 1.76 and 2.0 and the limit of quantification was found to be 1.87 and 1.48. The method was known to be accurate with the assay method. The % assay was found to be 97 and 98.12. The developed method was showed to a good accuracy and precision. The % RSD is for Omeprazole and Domperidone is 041 and 1.41. The Isocratic elution technique developed for the determination of Omeprazole and Domperidone ideally suited for rapid and routine analysis. This method shows that good reproducibility of the results. Furthermore this method was simple, sensitive, and accurate. Degradation studies were done, here the drug stability results were in the range of acceptance criteria 85–115%.[29]

AIM AND PLAN OF WORK

4. AIM AND PLAN OF WORK

4.1 AIM

To develop and validate a new method for the simultaneous determination of Ilaprazole and Domperidone in capsule dosage form by Ultra Performance Liquid Chromatography [UPLC].

4.2 OBJECTIVE

The literature review revealed that there are several methods reported for the estimation Ilaprazole and Domperidone alone or in combination with other drugs in their pharmaceutical dosage forms but none of the method available for the estimation of these drugs in the selected pharmaceutical dosage form by this intended method (UPLC).

It is common to administer two or more drugs in a single formulation and it may be to reduce the number of medicaments to be taken at a time for better patient compliance. It is a challenging task for the analyst to develop a simple analytical method for simultaneous estimation of multiple drug formulations with desired degree of accuracy and precision. In the present work attempts have been made to develop Rapid UPLC method of analysis for a simultaneous estimation of selected two drugs in capsule dosage form.

Hence on the basis of the literature survey, it was thought to develop a precise, accurate, simple, rapid and reliable method for estimation of Ilaprazole and Domperidone in capsules dosage form using the following technique of UPLC.

4.3 PLAN OF WORK

Plan of work was designed as follows:

I. Literature collection.

II. Study of physicochemical properties of drug (pH, pKa and solubility).

III. Procurement of chemicals and API.

IV. Method development and optimization of chromatographic conditions

- Selection of diluent
- Selection of wavelength
- Choice of chromatographic method
- Selection of initial separation
- Optimize the chromatographic variables.

V. Development of UPLC method for the quantification of Ilaprazole and Domperidonein capsules dosage form.

VI. Validation of proposed method as per ICH Q2 (R1) guidelines.

MATERIALS AND EQUIPMENTS

5. MATERIALS AND EQUIPMENTS

5.1 INSTRUMENTS USED

Table 5.1: List of instruments used during validation studies

Sl. No.	Instruments Name	Model
01	UV- Visible Spectrophotometer	Shimadzu 1800
02	UPLC with PDA detector	Thermo scientific
03	Analytical Balance	Metler
04	pH meter	Eutech
05	Ultra Sonicator	Remi Instrument Ltd
06	Pipettes and Burettes	Borosil (Class A)
07	Glass Beakers	Borosil
08	Measuring Cylinder	Borosil
09	Volumetric(Standard) Flask	Borosil (Class A)

5.2 DRUGS AND CHEMICALS USED

Ilaprazole and Domperidone was obtained from **Aeon Formulations Pvt. Ltd.**(Puducherry, India) as gift sample and marketed formulation **Ilavieon DSR capsules**(Ilaprazole 10 mg and Domperidone30mg) manufactured **by Aeon Formulations Pvt. Ltd.** was procured form local market of Chennai.

Table 5.2: List of chemicals used during validation studies

Sl No.	Materials	Manufacturer	Batch no/ Control No
01	Triethylamine	MERCK	SF3S630365
02	Ammonium acetate	MERCK	QE4Q641100
03	Phosphoric acid	MERCK	PM2P523951
04	Acetic acid	MERCK	CJ4C640904
05	Methanol	HIMEDIA	MET/1510032
06	Acetonitrile	MERCK	DL4DF41617
07	HPLC water	MILLI-Q	112871

ANALYTICAL METHOD DEVELOPMENT AND OPTIMIZATION

6. ANALYTICAL METHOD DEVELOPMENT AND OPTIMIZTION

A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modelling. The important factors, which are to be taken into account to obtain reliable quantitative analysis, are

- Careful sampling and sample preparation.
- Precise sample injection.
- Appropriate choice of the column.
- Choice of the operating conditions to obtain the adequate resolution of mixture.
- Reliable performance of the recording and data handling systems.
- Suitable integration/peak height measurement techniques.
- The mode of calculation best suited for the purpose.
- Validation of the developed method.

Stock Solution A(Ilaprazole)

Weighed accurately about 20.03 mg of Ilaprazole and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 400.6µg/ml.

Stock Solution B (Domperidone)

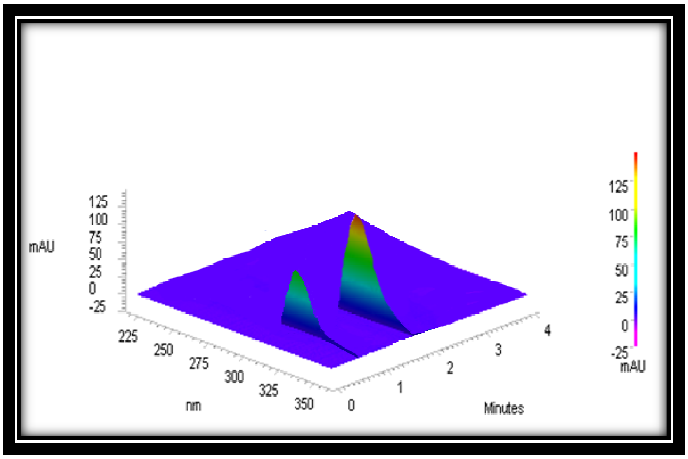
Weighed accurately about 61.18mg of Domperidone and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 1223.6µg /ml.

Standard Solution Preparation

1 ml of stock solution A and 1 ml of stock solution B were mixed in a 100 ml dried and cleaned standard flask. This was then diluted with 30 ml of methanol. The volume was made up to 100 ml with same solvent. This was marked and labelled as standard solution which contains 4.006 µg/ml of Ilaprazole and 12.236µg/ml of Domperidone. Resulting solution was then filtered with 0.45 µ membrane filter.

Selection Of Wavelength (λ_{max})

The sensitivity of the UPLC method that uses PDA detection depends upon the proper selection of the wavelength. An ideal wavelength is one that gives good response for the drugs to be detected. In the entire UV visible region both the drugs were strongly absorbed at 288 nm. So this wavelength was selected for further studies.



Wavelength Selection of Combined Drugs (Domperidone and Ilaprazole)

6.1 Development of RP-UPLC method for Ilaprazole and Domperidone

Trial 1

Chromatographic condition

Mobile phase	:	0.5% W/V of Ammonium acetate : Methanol (50:50)
Column	:	C18(50 X 2.1 mm, 1.9 µm)
Diluent	:	Methanol
Flow rate	:	0.4ml/min
Run time	:	5 min
Injection volume	:	5µl
Column temperature	:	Ambient
PDA Detection	:	288 nm
Instrument Make	:	Thermo
Mode	:	Accela 1250
Software	:	Chroquest 5.0

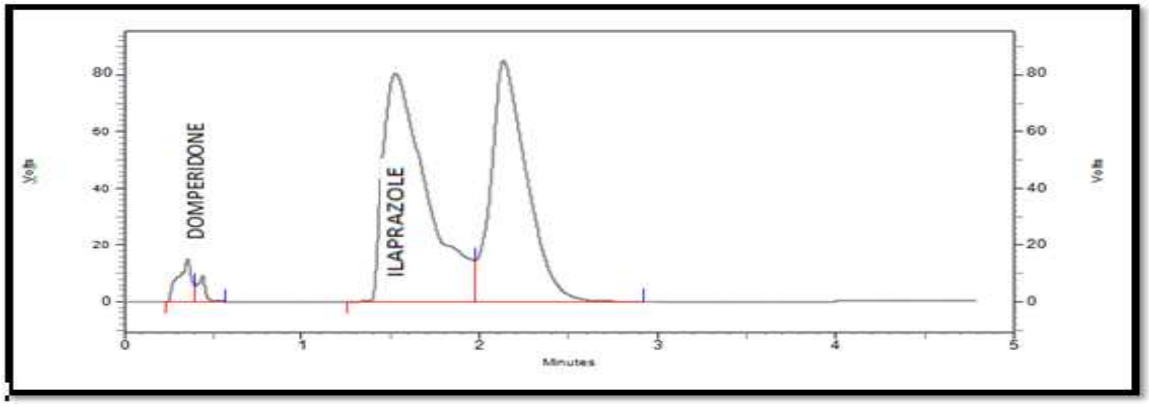


Figure 6.1: Trial chromatogram 1 for Ilaprazole and Domperidone

Observation: From the above chromatogram it was observed that the Ilaprazole peak was splitted and the shape of Domperidone peak is not good. Hence this trial is not suitable.

Trial 2

Chromatographic condition

Mobile phase	:	Phosphoric acid (pH adjusted to 3.5 with Triethylamine): Methanol (60:40)
Column	:	C18(50 X 2.1 mm, 1.9 µm)
Diluent	:	Methanol
Flow rate	:	0.4ml/min
Run time	:	4 min
Injection volume	:	10µl
Column temperature	:	Ambient
PDA Detection	:	288 nm
Instrument Make	:	Thermo
Mode	:	Accela 1250
Software	:	Chroquest 5.0

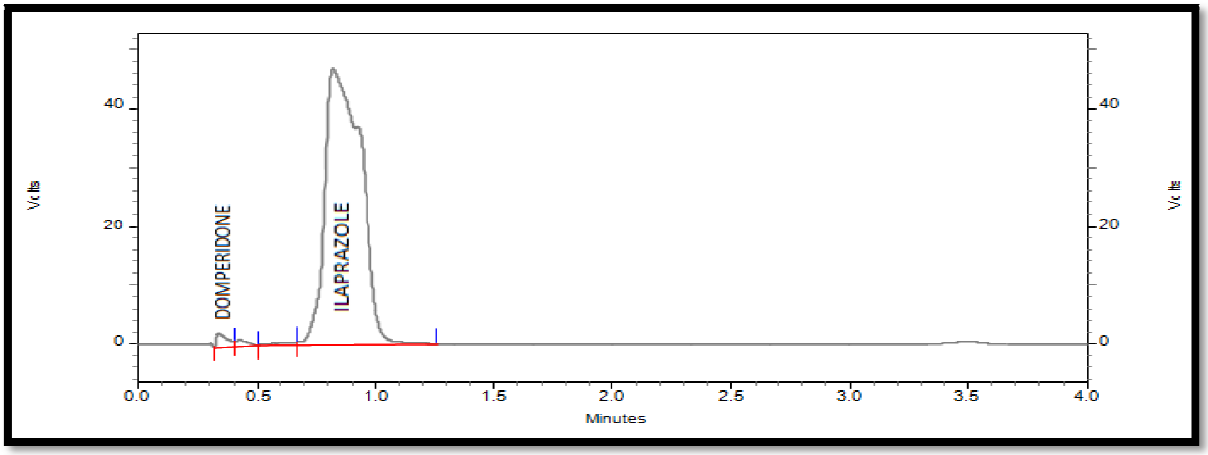


Figure 6.2: Trial chromatogram 2 for Ilaprazole and Domperidone

Observation: From the above peak it was observed that Ilaprazole peak was splitted and the shape of Domperidone peak is not good. Hence this trial is not suitable.

TRIAL 3

Chromatographic condition

Mobile phase	:	Water : Methanol(30:20) finally adjust the pH to 5.0 with Triethylamine
Column	:	C18(50 X 2.1 mm, 1.9 µm)
Diluent	:	Methanol
Flow rate	:	0.4ml/min
Run time	:	4 min
Injection volume	:	10µl
Column temperature	:	Ambient
PDA Detection	:	288 nm
Instrument Make	:	Thermo
Mode	:	Accela 1250
Software	:	Chroquest 5.0

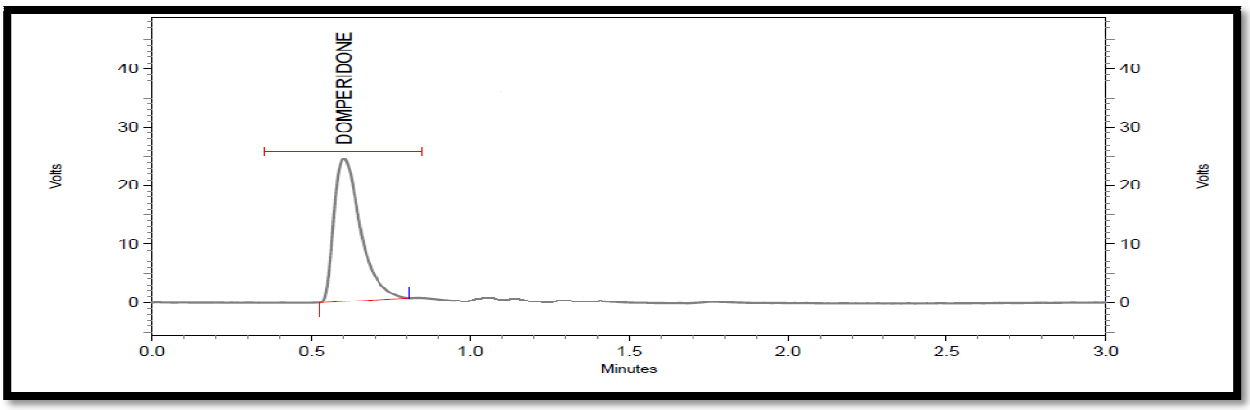


Figure 6.3: Trial chromatogram 3 for Ilaprazole and Domperidone

Observation: From the above chromatogram it was observed that the Ilaprazole peak is not eluted. Hence this trial is not suitable.

Trial 4

Chromatographic condition

Mobile phase	:	Water : Methanol : ACN : Acetic Acid (30:20:50:0.3) finally adjust the pH to 5.0 with Triethylamine
Column	:	C18(50 X 2.1 mm, 1.9 µm)
Diluent	:	Methanol
Flow rate	:	0.4ml/min
Run time	:	3 min
Injection volume	:	10µl
Column temperature	:	Ambient
PDA Detection	:	288 nm
Instrument Make	:	Thermo
Mode	:	Accela 1250
Software	:	Chroquest 5.0

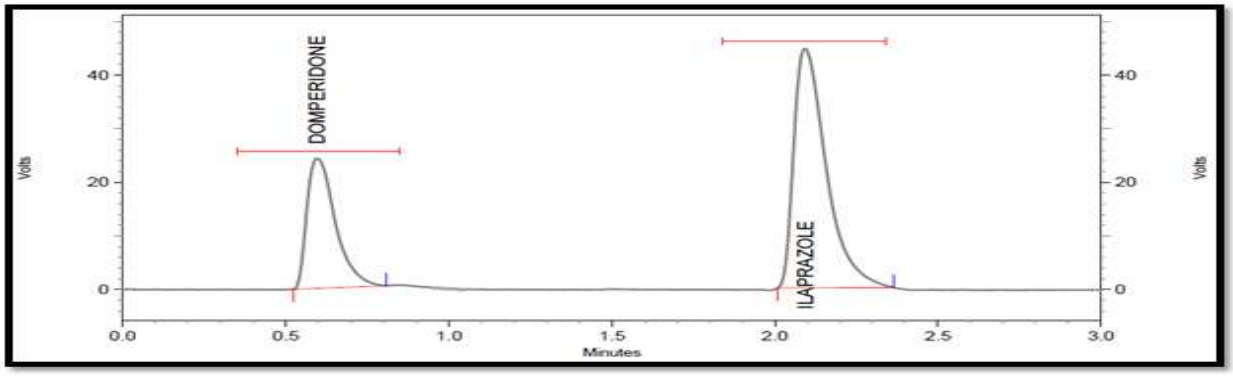


Figure 6.4: Trial chromatogram 4 for Ilaprazole and Domperidone

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.598	145852	32.27	2249	0.00	1.70
IPZ	2.072	306090	67.73	2589	16.81	1.85
Total		451942	100.00			

Observation: From the above chromatogram it was observed that DPD and IPZ are well separated. Retention time of DPD and IPZ are 0.598 and 1.072 minute respectively. So this method was found as optimized method and selected for further analysis.

Optimized Analytical Method

Four trials were performed by changing the different parameters like flow rate, injection volume mobile phase etc. From the above four trails it has been observed that trial 4 is optimized method to carry out the validation of IPZ and DPD in marketed capsule formulation. Optimized chromatographic parameters are given below.

Chromatographic condition

Mobile phase	:	Water : Methanol : ACN : Acetic Acid (30:20:50:0.3) finally adjust the pH to 5.0 with Triethylamine
Column	:	C18(50 X 2.1 mm, 1.9 µm)
Diluent	:	Methanol
Flow rate	:	0.4ml/min
Run time	:	3 min
Injection volume	:	10µl
Pump Mode	:	Isocratic
Column temperature	:	Ambient
PDA Detection	:	288 nm
Instrument Make	:	Thermo
Mode	:	Accela 1250
Software	:	Chroquest 5.0

6.2 Quantitative Estimation of Ilaprazole and Domperidone

System suitability

This parameter was tested by giving five replicate injections of the standard solution to check the system suitability parameters like asymmetry, theoretical plates, tailing factor etc. Weight taken for

stock solution A & B were 51.28 mg of IPZ and 12.65 mg of DPD respectively. Suitability parameters were calculated and given in table6.1& 6.2.

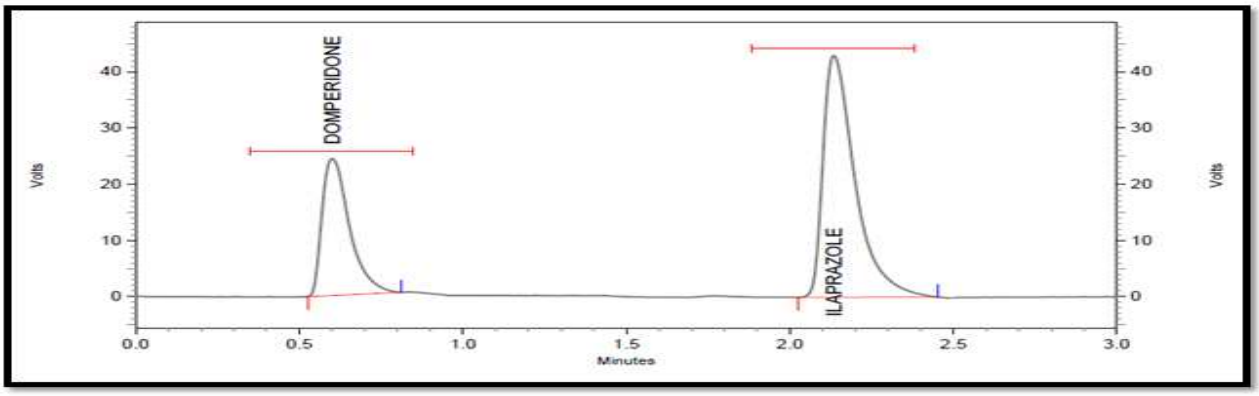


Figure 6.5: Chromatogram of System Suitability of Standard, Replicate Injection 01

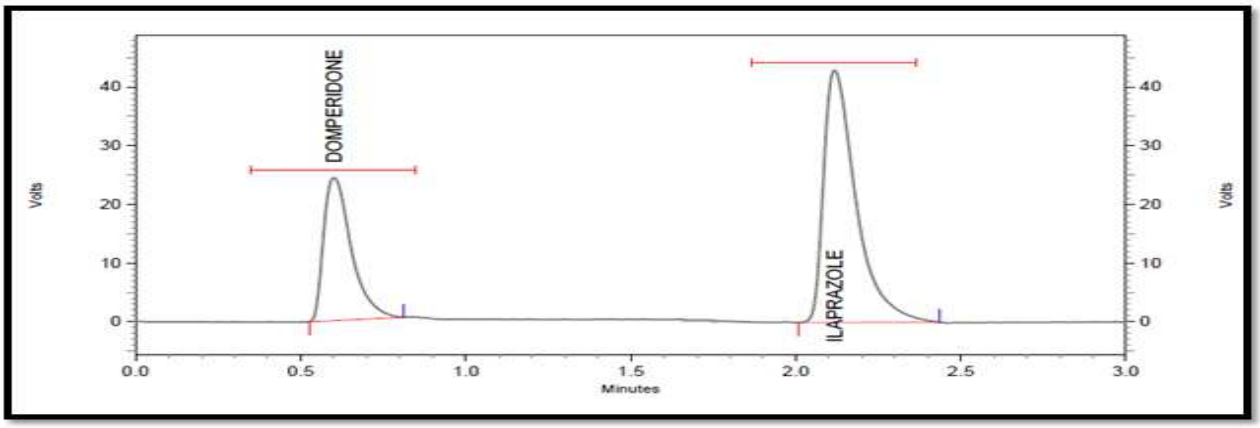


Figure 6.6: Chromatogram of System Suitability of Standard, Replicate Injection 02

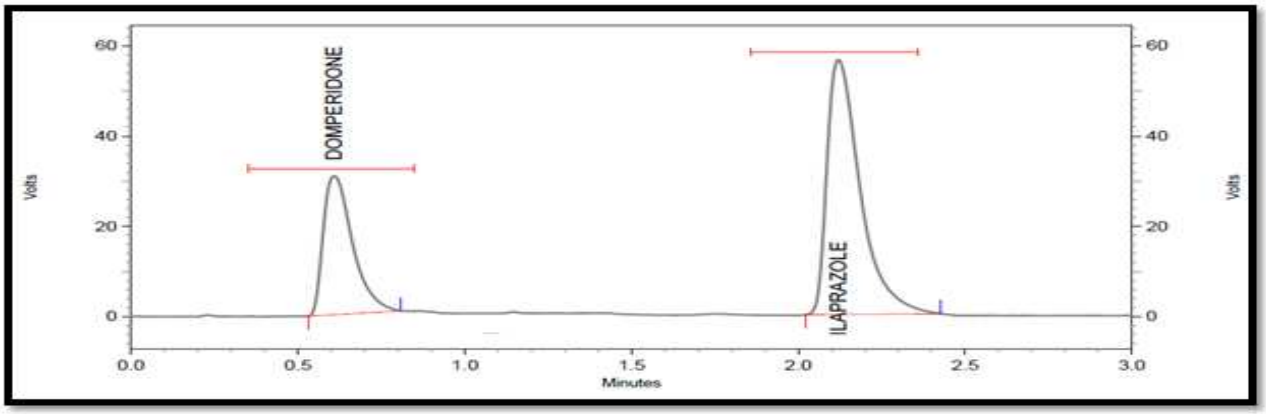


Figure 6.7: Chromatogram of System Suitability of Standard, Replicate Injection 03

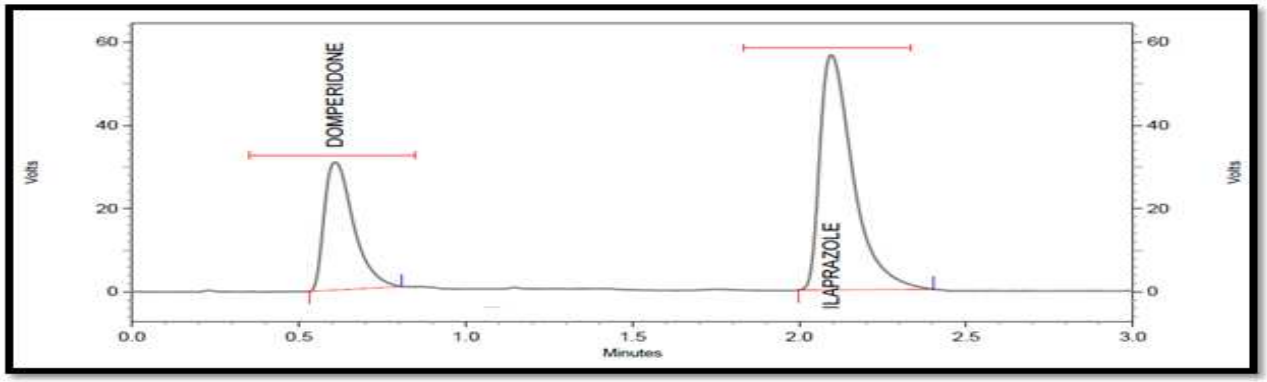


Figure 6.8: Chromatogram of System Suitability of Standard, Replicate Injection 04

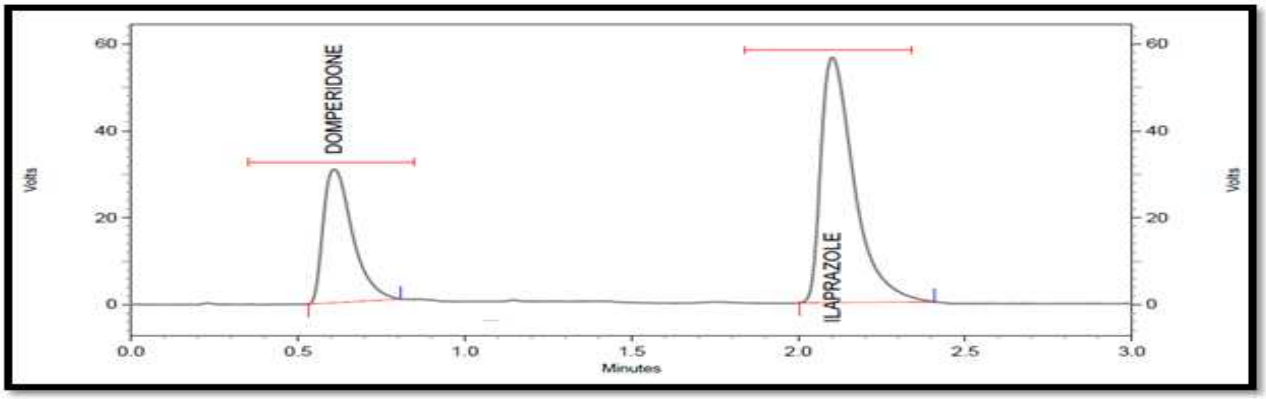


Figure 6.9: Chromatogram of System Suitability of Standard, Replicate Injection 05

Table 6.1: Results of System Suitability Domperidone

INJECTION ID	RETENTION TIME	AREA	THEORETICAL PLATES (USP)	ASYMMETRY
1	0.600	149623	2264	1.69
2	0.598	149725	2264	1.68
3	0.600	149841	2263	1.66
4	0.600	148468	2268	1.73
5	0.602	149101	2265	1.71
AVERAGE		149352	2264.80	1.69
STD DEV		569		
% RSD		0.381		

Table 6.2: Results of System Suitability Ilaprazole

INJECTION ID	RETENTION TIME	AREA	THEORETICAL PLATES (USP)	ASYMMETRY
1	2.077	306754	2624	1.82
2	2.072	309482	2609	1.80
3	2.077	309371	2616	1.77
4	2.067	309673	2607	1.88
5	2.077	308925	2644	1.84
AVERAGE		308841	2620	1.82
STD DEV		1199		
% RSD		0.388		

Acceptance Criteria : Asymmetry NMT 2.0

Theoretical plates NLT 2000

RSD of area NMT 2.0

Report : The all system suitability parameters for IPZ and DPD were found within limits.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. To demonstrate the linearity of analytes over the range 80% to 120% of target concentration five different concentration solutions of the DPD & IPZ (80%, 90%, 100%, 110%, and 120%) prepared and take injections in UPLC by the means of auto injector.

Procedure

Stock Solution A (Ilaprazole)

Weighed accurately about 20 mg of Ilaprazole and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 400µg/ml.

Stock Solution B (Domperidone)

Weighed accurately about 60 mg of Domperidone and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 1200 µg /ml.

Actual weight taken,

For stock solution A (Ilaprazole) = 20.03 mg

For stock solution B (Domperidone) = 61.18 mg

Preparation of 80% Solution:

Dilute 0.8 ml of stock solution A and 0.8 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

Preparation of 90% Solution:

Dilute 0.9 ml of stock solution A and 0.9 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

Preparation of 100% Solution:

Dilute 1 ml of stock solution A and 1 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

Preparation of 110% Solution:

Dilute 1.1 ml of stock solution A and 1.1 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

Preparation of 120% Solution:

Dilute 1.2 ml of stock solution A and 1.2 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

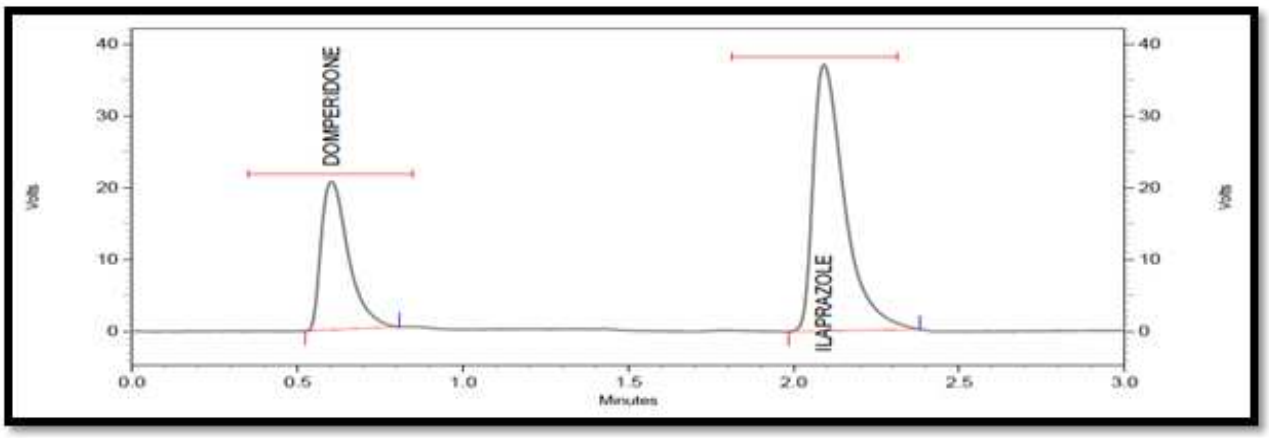


Figure 6.10: Chromatogram of linearity solution (80%)

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.603	115657	32.29	2282	0.00	1.73
IPZ	2.078	242560	67.71	2695	16.76	1.85
Total		358217	100.00			

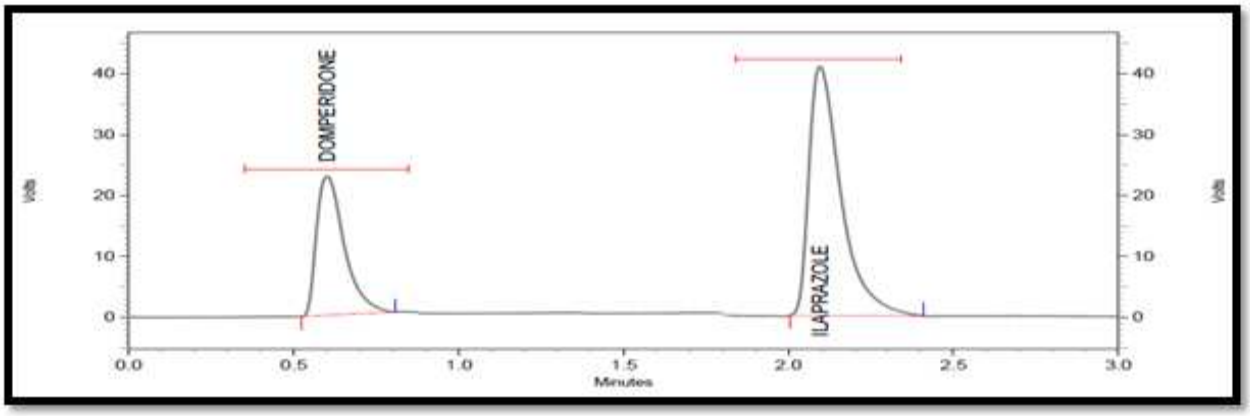


Figure 6.11: Chromatogram of linearity solution (90%)

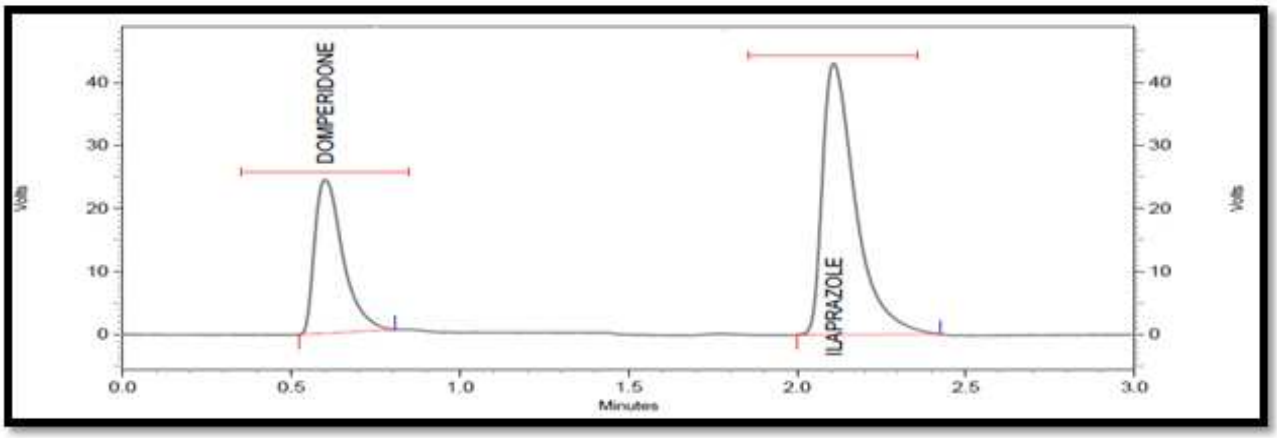


Figure 6.12: Chromatogram of linearity solution (100%)

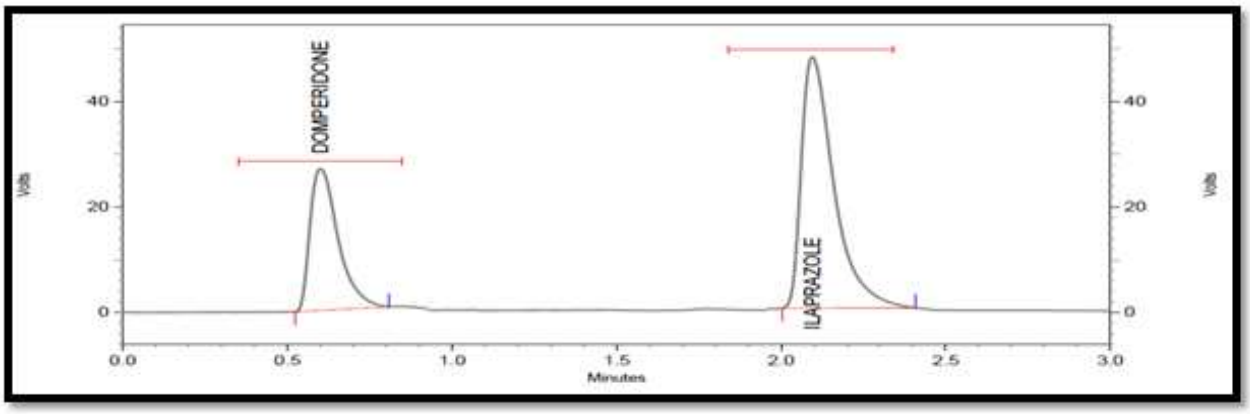


Figure 6.13: Chromatogram of linearity solution (110%)

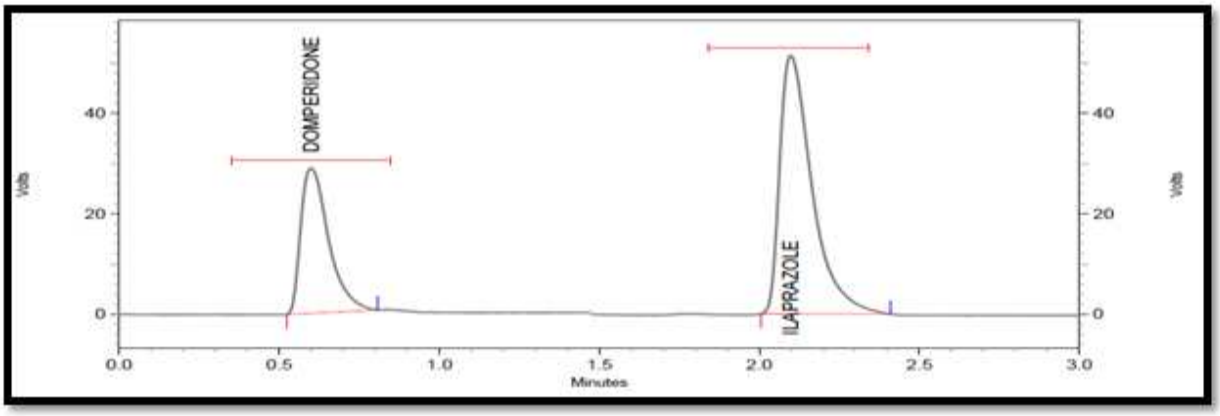


Figure 6.14: Chromatogram of linearity solution (120%)

Table 6.3: Linearity results for Domperidone and Ilaprazole

SL. NO	LINEARITY LEVEL	DOMPERIDONE (DPD)		ILAPRAZOLE (IPZ)	
		CONCENTRATION (µg/ml)	AREA	CONCENTRATION (µg/ml)	AREA
1	**80%**	9.79	115657	3.20	242560
2	**90%**	11.01	131110	3.61	275505
3	**100%**	12.24	145029	4.01	302861
4	**110%**	13.46	159996	4.41	331534
5	**120%**	14.68	174431	4.81	362008
Correlation coefficient		0.999		0.999	
Slope		11973		73371	

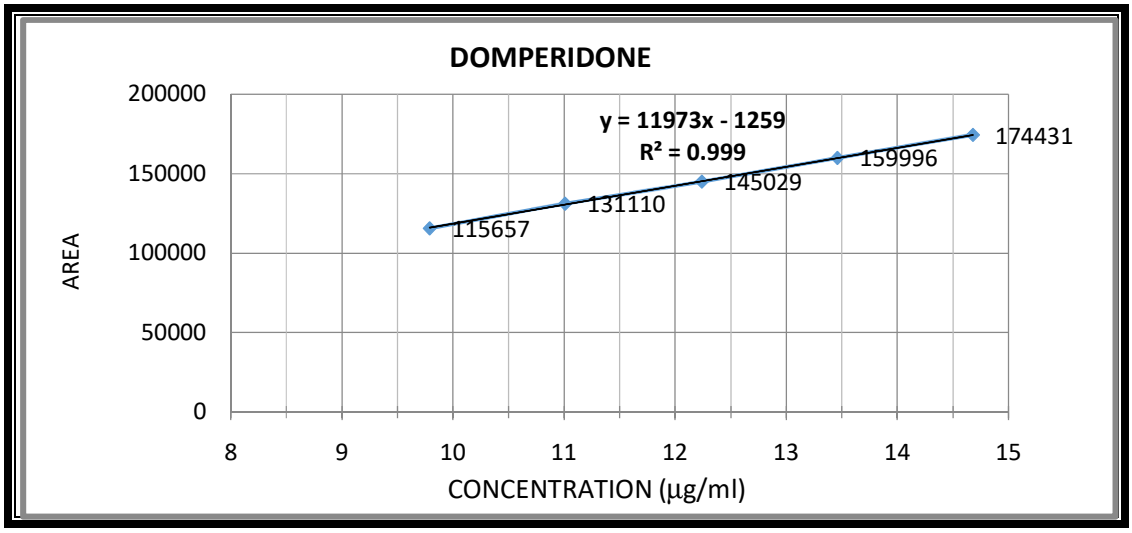


Figure 6.15: Calibration graph of Domperidone

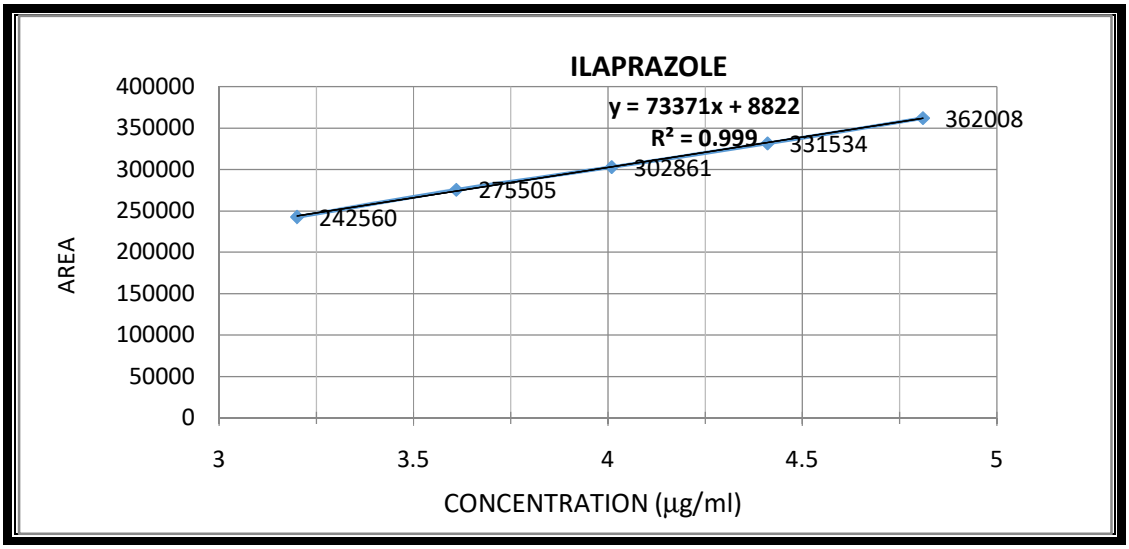


Figure 6.16: Calibration graph of Ilaprazole

Acceptance criteria: Correlation coefficient ≥ 0.997

Report: The relationship between the concentration and the peak response of Domperidone (DPD) and Ilaprazole (IPZ) were found linear in the specific range and regression coefficient for both Domperidone and Ilaprazole was found to be 0.999.

Assay of Ilaprazole and Domperidone in Capsule Dosage Form

Preparation of Standard Solution

Stock Solution A (Ilaprazole)

Weighed accurately about 20 mg of Ilaprazole and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 400µg/ml.

Stock Solution B (Domperidone)

Weighed accurately about 60 mg of Domperidone and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 1200µg /ml.

Standard Solution Preparation

1 ml of stock solution A and 1 ml of stock solution B were mixed in a 100 ml dried and cleaned standard flask. This was then diluted with 30 ml of methanol. The volume was made up to 100 ml with same solvent. This was marked and labelled as standard solution which contains 4.006 µg /ml of Ilaprazole and 12.00µg/ml of Domperidone. Resulting solution was then filtered with 0.45 µ membrane filter.

Preparation of Sample Solution

Twenty capsules were accurately weighed and the content of the shell were removed completely to a clean and dried petri plate.Clean inside of the shells and the empty capsule shells were weighed accurately. Average weight of the powder for 20 capsules was calculated. A quantity of powder weigh equivalent to 10 mg of IPZ and 30 mg of DPD was

weighed and transferred to a 25 ml volumetric flask and sufficient diluent was added to dissolve it. Then the solution was sonicated for 10 min. Final volume was adjusted with the same solvent and filtered through 0.45 µ membrane filter. 1 ml of the solution was pipetted out in a 100 ml dried and cleaned standard flask. This was then diluted with 30 ml of methanol. The volume was made up to 100 ml with same solvent. Resulting solution was then filtered through 0.45 µ membrane filter. The sample solution contains 4µg/ml of Ilaprazole and 12 µg/ml of Domperidone.

Procedure:

Standard and sample solution were loaded on vial and injected into the chromatographic system with the help of auto injector separately. Injection has to be carried out by 5 replication of standard followed by sample.

Average weight of 20 capsules = 320.0 mg

Weight taken = 316.18 mg

Label claim: 10 mg of Ilaprazole

30 mg of Domperidone

Purity of Working Standard purity

Ilaprazole : 99.48%

Domperidone : 99.22%

Weight taken for standard Solution

Ilaprazole : 20.03 mg

Domperidone : 61.18 mg

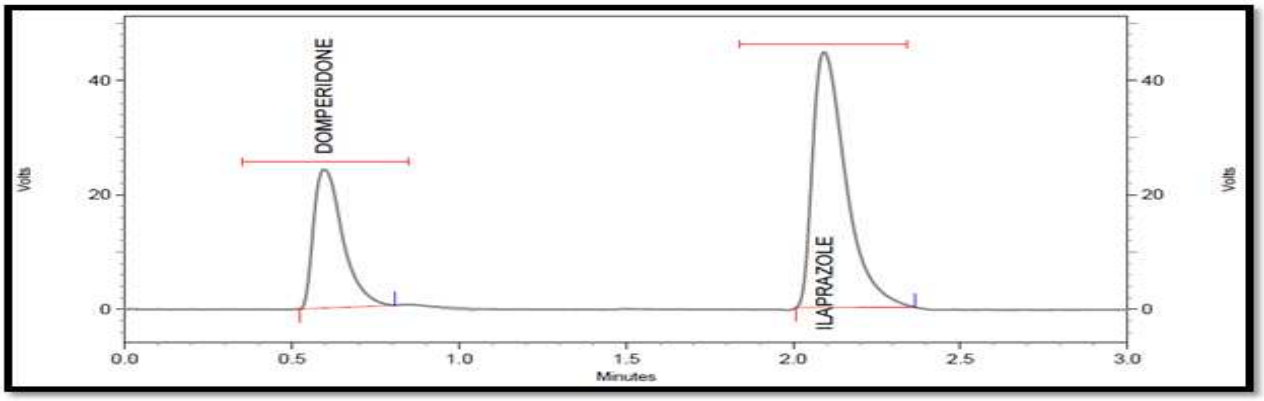


Figure 6.17: Chromatogram of Sample (Marketed Capsule)

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.598	149725	32.27	2249	0.00	1.70
IPZ	2.072	306090	67.73	2589	16.75	1.85
Total		451942	100.00			

Calculation:

The amount of Ilaprazole and Domperidone present in each capsule were calculated by using the following formula:

Amount present

$$= \frac{\text{Sample Area}}{\text{Standard Area}} \text{ X } \frac{\text{Standard weight}}{\text{Standard Dilution}} \text{ X } \frac{\text{Sample Dilution}}{\text{Sample weight}} \text{X} \frac{\text{WS purity}}{100} \text{ X Average weight}$$

$$\textbf{Assay} = \frac{\text{Amount present}}{\text{Label Claim}} \text{ X } 100$$

Table 6.4: Tabular column for the assay of Capsule sample

Sl. No	Drug Name	Sample Area	Standard Area	Label claim (mg)	Amount Present (mg)	Assay %
1	DPD	145831	149352	30	29.99	99.98
2	IPZ	306090	308841	10	10.00	100.00

Report: The percentage purity of Domperidone and Ilaprazole were calculated in capsule dosage form and it was found to be 102.53 % and 100.00 % respectively.

VALIDATION OF DEVELOPED METHOD

7. VALIDATION OF DEVELOPED METHOD

7.1 PREPARATION OF SOLUTION

7.1.1 Preparation of mobile phase

Water, Methanol, Acetonitrile and Acetic acid were mixed in the ratio of 30:20:50:0.3andthe pH was adjusted to 5.0 with Triethylamine. Sonicated the solution by the help of ultra sonicator for 20 minutes and filtered with 0.45 µ membrane filter.

7.1.2 Preparation of working standard solution

Stock Solution A (Ilaprazole)

Weighed accurately about 20 mg of Ilaprazole and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 400µg/ml.

Stock Solution B (Domperidone)

Weighed accurately about 60mg of Domperidone and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 1200µg /ml.

7.1.3 Standard Solution Preparation

1 ml of stock solution A and 1 ml of stock solution B were mixed in a 100 ml dried and cleaned standard flask. This was then diluted with 30 ml of methanol. The volume was made up to 100 ml with same solvent. This was marked and labelled as standard stock solution which contains

4.006µg /ml of Ilaprazole and 12.00µg/ml of Domperidone. Resulting solution was then filtered with 0.45 µ membrane filter.

6.1.4 Preparation of Sample solutions

Twenty capsules were accurately weighed and the content of the shell were removed completely to a clean and dried petri plate.Clean inside of the shells and the empty capsule shells were weighed accurately. Average weight of the powder for 20 capsules was calculated.A quantity of powder weigh equivalent to 10mg of IPZ and 30 mg of DPD was weighed and transferred to a25 ml volumetric flask and sufficient diluent was added to dissolve it. Then the solution was sonicated for 10 min. Final volume was adjusted with the same solvent and filtered through 0.45 µ membrane filter. 1 ml of the solution was pipetted out in a 100 ml dried and cleaned standard flask. This was then diluted with 30 ml of methanol. The volume was made up to 100 ml with same solvent. Resulting solution was then filtered through 0.45 µ membrane filter.

The sample solution contains 4µg/ml of Ilaprazole and 12µg/ml of Domperidone. The amount of Ilaprazole and Domperidone present in each capsule were calculated by using the following formula:

$$\text{Amount present} = \frac{\text{Sample Area}}{\text{Standard Area}} \text{ X } \frac{\text{Standard weight}}{\text{Standard Dilution}} \text{ X } \frac{\text{Sample Dilution}}{\text{Sample weight}} \text{X} \frac{\text{Percentage purity}}{100} \text{ X Average weight}$$

$$\% \text{ Amount present} = \frac{\text{Amount present}}{\text{Label Claim}} \text{ X } 100$$

7.2 ANALYTICAL METHOD VALIDATION

The developed method was validated according to ICH guidelines

Method validation of Ilaprazole and Domperidone

The mobile phase was prepared and all parameters were set as per the above optimized method.

7.2.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the method was evaluated with regard to interference due to presence of any other excipients. The figure shows that drug was clearly separated from its excipients. Thus, the UPLC method presented in this study is selective.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Identification of Ilaprazole and Domperidone: Solutions of standard and Sample were prepared as per test method and injected into the chromatographic system.

Acceptance criteria: Chromatogram of Standard and sample should be identical with near /same retention time.

Blank interference: A study to establish the interference, blank detection was conducted. Mobile phase was injected as per the test method.

Acceptance criteria: Chromatogram of blank did not show any peak at the retention time of analyte peak.

The blank, standard solution and sample solution were loaded on vial and chromatogram was recorded.

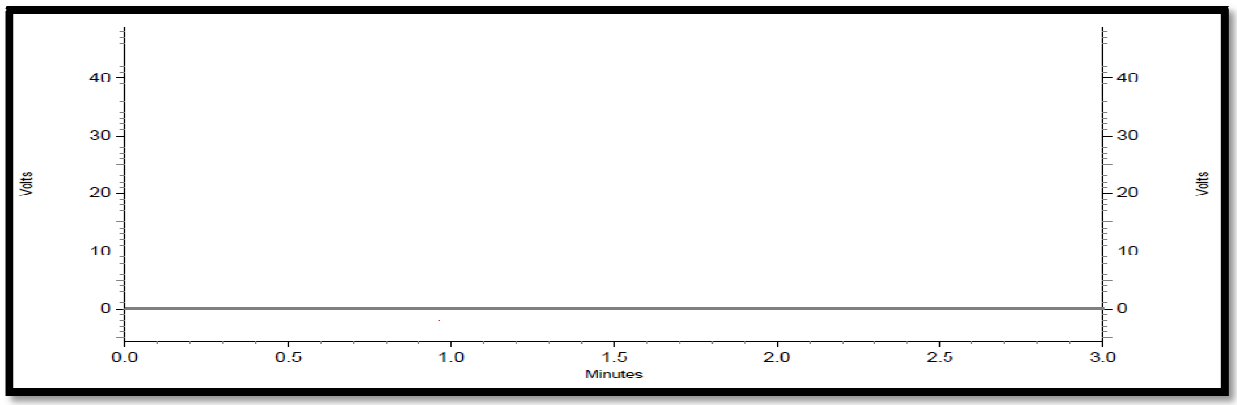


Figure 7.1: Chromatogram of blank

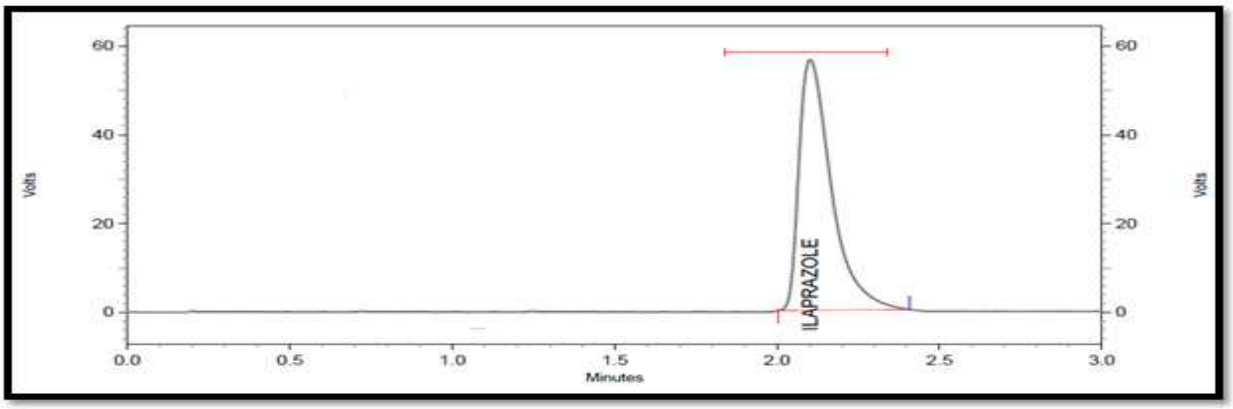


Figure 7.2: Chromatogram of Ilaprazole (WS)

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
IPZ	2.077	332450	100	2570	0.00	1.82

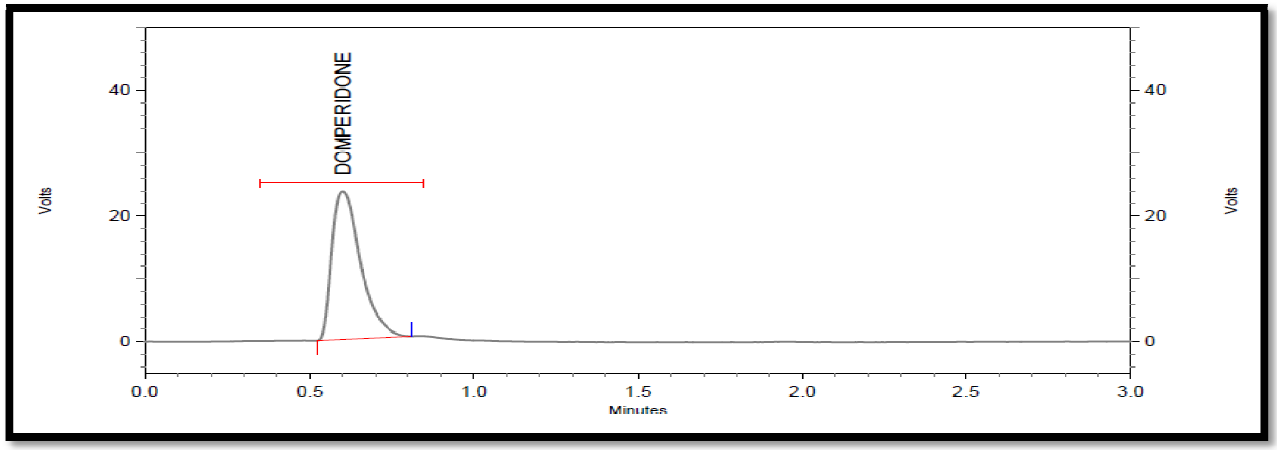


Figure 7.3: Chromatogram of Domperidone (WS)

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.600	143549	100	2244	0.00	1.68

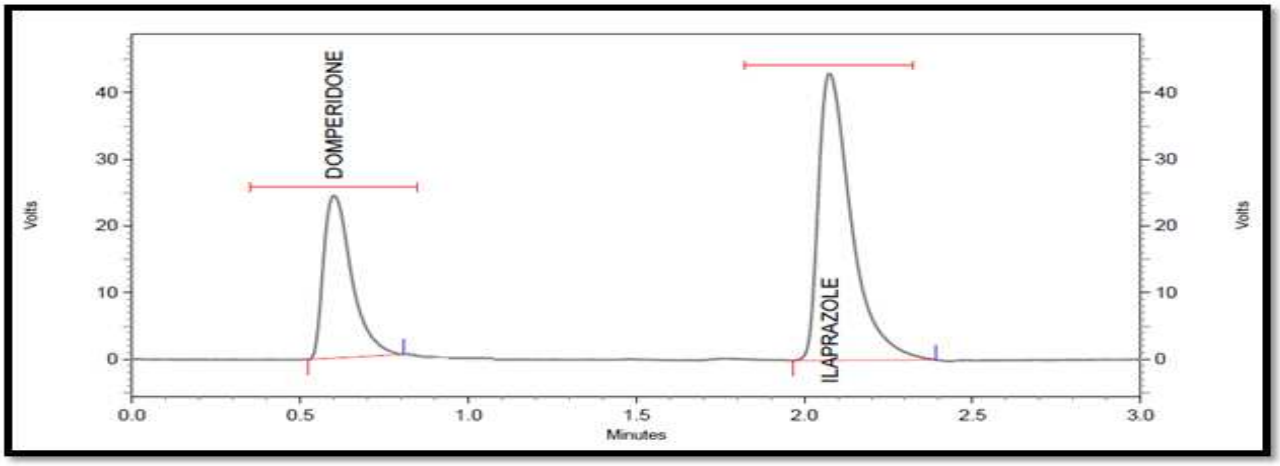


Figure 7.4: Chromatogram of sample solution

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.600	149841	32.63	2263	0.00	1.66
IPZ	2.077	309371	67.37	2616	16.78	1.77
Total		459212	100.00			

Report : Chromatogram of blank did not show any peak at the retention time of analytepeak and retention time of standard and sample were identical.

7.2.2 System suitability

This parameter was tested by giving five replicate injections of the standard solution to check the system suitability parameters like asymmetry, theoretical plates, tailing factor etc. Weight taken for stock solution A & B were 51.28 mg of IPZ and 12.65 mg of DPD respectively. Suitability parameters were calculated and given in table 7.1 & 7.2.

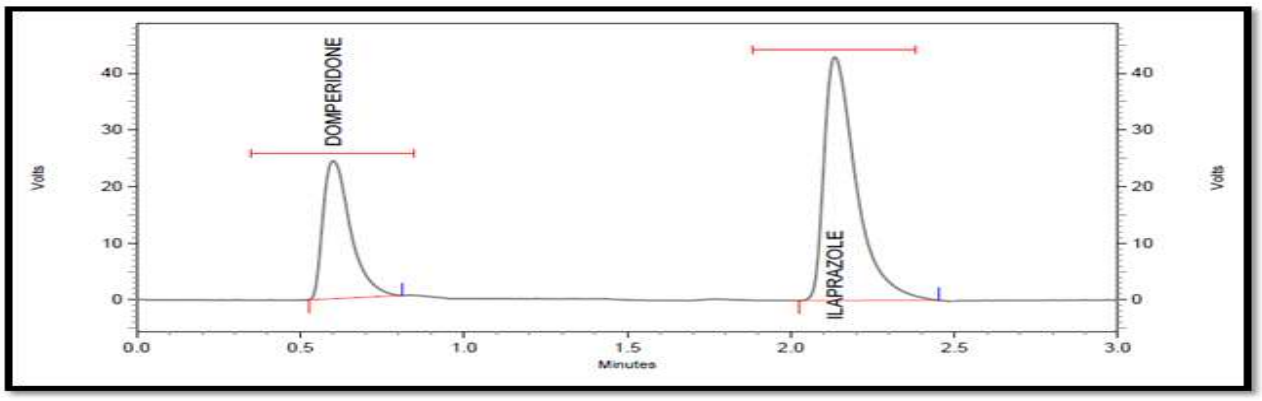


Figure 7.5: Chromatogram of System Suitability of Standard, Replicate Injection 01

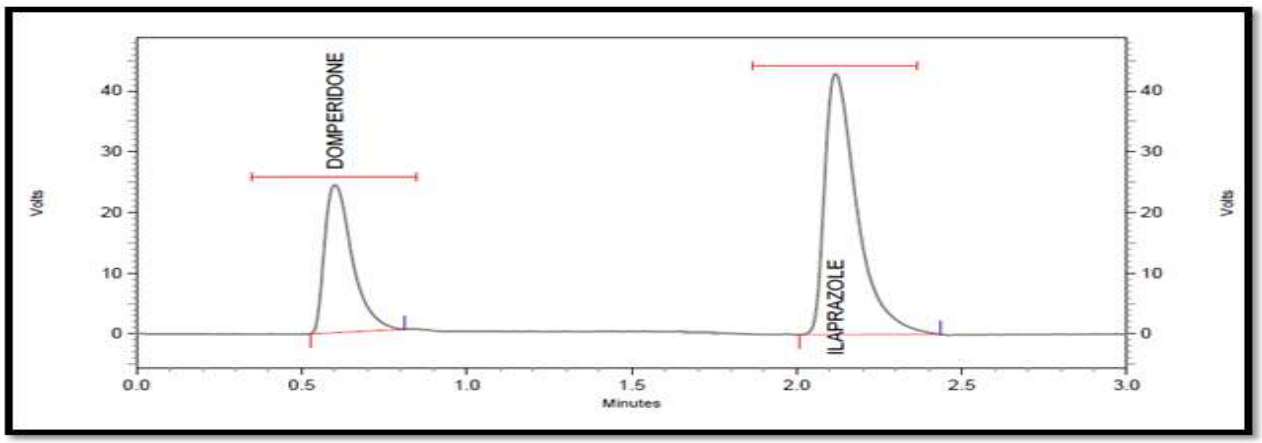


Figure 7.6: Chromatogram of System Suitability of Standard, Replicate Injection 02

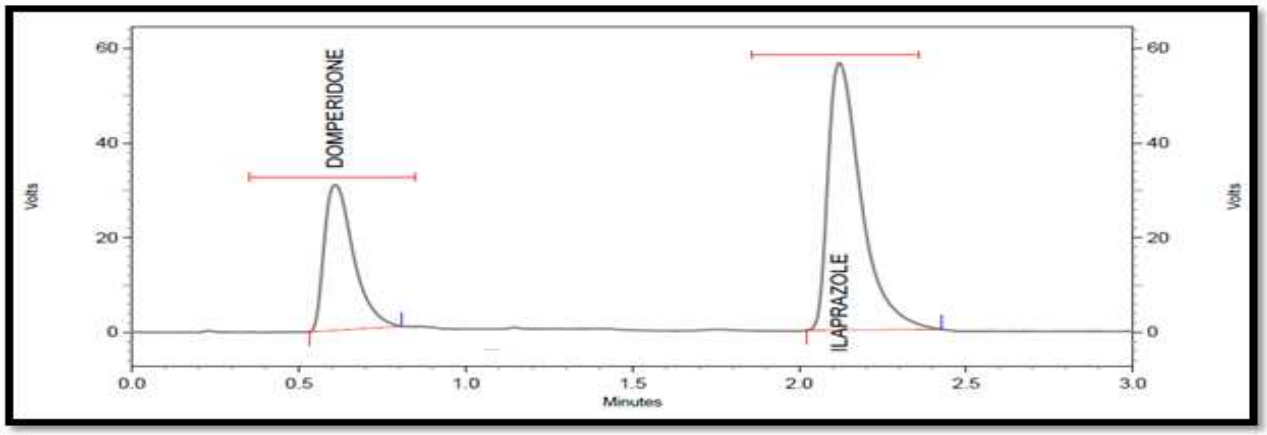


Figure 7.7: Chromatogram of System Suitability of Standard, Replicate Injection 03

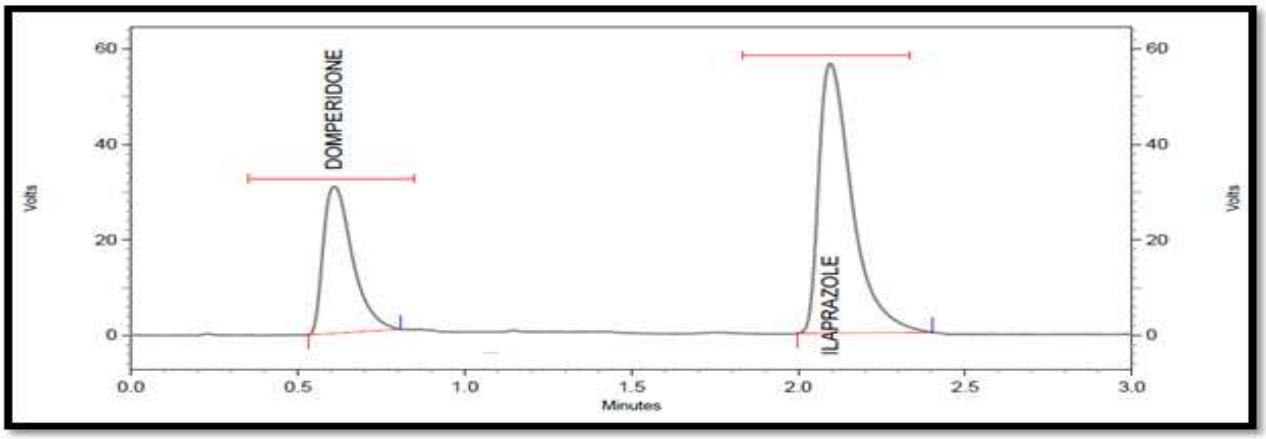


Figure 7.8: Chromatogram of System Suitability of Standard, Replicate Injection 04

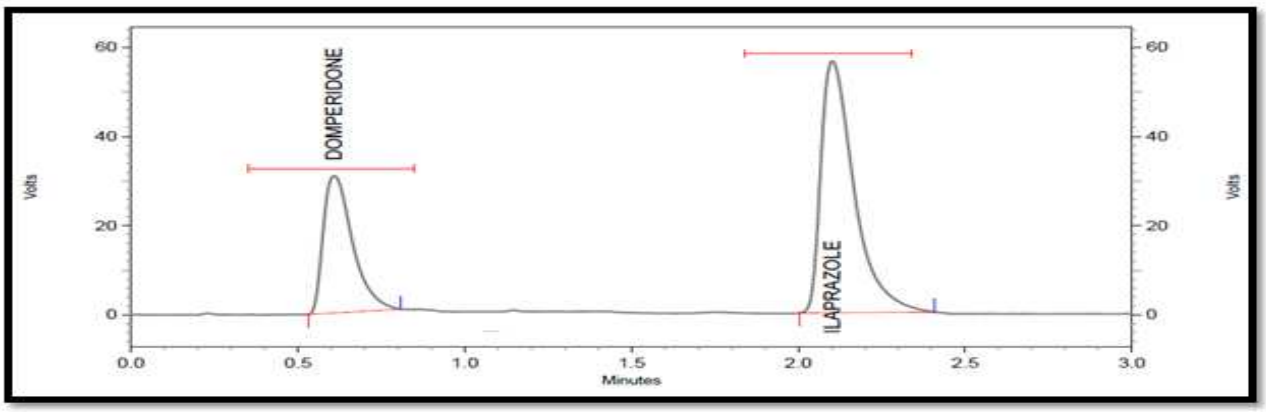


Figure 7.9: Chromatogram of System Suitability of Standard, Replicate Injection 05

Table 7.1: Results of System Suitability Domperidone

INJECTION ID	RETENTION TIME	AREA	THEORETICAL PLATES (USP)	ASYMMETRY
1	0.600	149623	2264	1.69
2	0.598	149725	2264	1.68
3	0.600	149841	2263	1.66
4	0.600	148468	2268	1.73
5	0.602	149101	2265	1.71
AVERAGE		149352	2264.80	1.69
STD DEV		569		
% RSD		0.381		

Table 7.2: Results of System Suitability Ilaprazole

INJECTION ID	RETENTION TIME	AREA	THEORETICAL PLATES (USP)	ASYMMETRY
1	2.077	306754	2624	1.82
2	2.072	309482	2609	1.80
3	2.077	309371	2616	1.77
4	2.067	309673	2607	1.88
5	2.077	308925	2644	1.84
AVERAGE		308841	2620	1.82
STD DEV		1199		
% RSD		0.388		

Acceptance Criteria : Asymmetry NMT 2.0

Theoretical plates NLT 2000

RSD of area NMT 2.0

Report : The all system suitability parameters for IPZ and DPD were found within limits.

7.2.3 Assay of Ilaprazole and Domperidone in Capsule Dosage Form

7.2.3.1 Preparation of Standard Solution

Stock Solution A (Ilaprazole)

Weighed accurately about 20 mg of Ilaprazole and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 400µg/ml.

Stock Solution B (Domperidone)

Weighed accurately about 60mg of Domperidone and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was

sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 1200µg /ml.

Standard Solution Preparation

1 ml of stock solution A and 1 ml of stock solution B were mixed in a 100 ml dried and cleaned standard flask. This was then diluted with 30 ml of methanol. The volume was made up to 100 ml with same solvent. This was marked and labelled as standard solution which contains 4.006 µg /ml of Ilaprazole and 12.00µg/ml of Domperidone. Resulting solution was then filtered with 0.45 µ membrane filter.

7.2.3.2 Preparation of Sample Solution

Twenty capsules were accurately weighed and the content of the shell were removed completely to a clean and dried petri plate.Clean inside of the shells and the empty capsule shells were weighed accurately. Average weight of the powder for 20 capsules was calculated. A quantity of powder weigh equivalent to 10 mg of IPZ and 30 mg of DPD was weighed and transferred to a 25 ml volumetric flask and sufficient diluent was added to dissolve it. Then the solution was sonicated for 10 min. Final volume was adjusted with the same solvent and filtered through 0.45 µ membrane filter. 1 ml of the solution was pipetted out in a 100 ml dried and cleaned standard flask. This was then diluted with 30 ml of methanol. The volume was made up to 100 ml with same solvent. Resulting solution was then filtered through 0.45 µ membrane filter. The sample solution contains 4µg/ml of Ilaprazole and 12 µg/ml of Domperidone.

Procedure:

Standard and sample solution were loaded on vial and injected into the chromatographic system with the help of auto injector separately. Injection has to be carried out by 5 replication of standard followed by sample.

Average weight of 20 capsules= 320.0 mg

Weight taken = 316.18mg

Label claim: 10 mg of Ilaprazole

30 mg of Domperidone

Purity of Working Standard purity

Ilaprazole : 99.48%

Domperidone : 99.22%

Weight taken for standard Solution

Ilaprazole : 20.03 mg

Domperidone : 61.18 mg

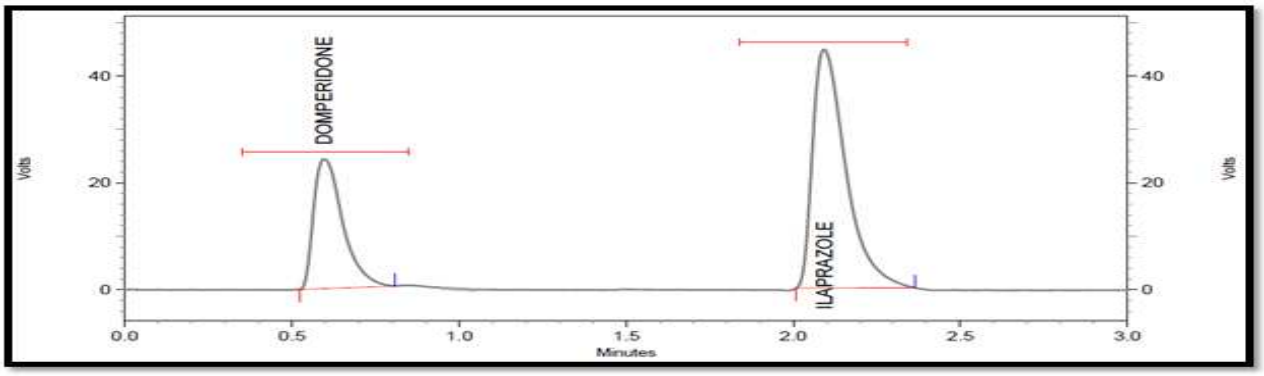


Figure 7.10: Chromatogram of Sample (Marketed Capsule)

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.598	149725	32.27	2249	0.00	1.70
IPZ	2.072	306090	67.73	2589	16.75	1.85
Total		451942	100.00			

Calculation:

The amount of Ilaprazole and Domperidone present in each capsule were calculated by using the following formula:

Amount present

$$= \frac{\text{Sample Area}}{\text{Standard Area}} \text{ X } \frac{\text{Standard weight}}{\text{Standard Dilution}} \text{ X } \frac{\text{Sample Dilution}}{\text{Sample weight}} \text{X} \frac{\text{WS purity}}{100} \text{ X Average weight}$$

$$\textbf{Assay} = \frac{\text{Amount present}}{\text{Label Claim}} \text{ X } 100$$

Table 7.3: Tabular column for the assay of Capsule sample

Sl. No	Drug Name	Sample Area	Standard Area	Label claim (mg)	Amount Present (mg)	Assay %
1	DPD	145831	149352	30	29.99	99.98
2	IPZ	306090	308841	10	10.00	100.00

Report: The percentage purity of Domperidone and Ilaprazole were calculated in capsule dosage form and it was found to be 102.53 % and 100.00 % respectively.

7.2.4 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. To demonstrate the linearity of analytes over the range 80% to 120% of target concentration five different concentration solutions of the DPD & IPZ (80%, 90%, 100%, 110%, and 120%) prepared and take injections in UPLC by the means of auto injector.

7.2.4.1 Procedure

Stock Solution A (Ilaprazole)

Weighed accurately about 20 mg of Ilaprazole and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 400µg/ml.

Stock Solution B (Domperidone)

Weighed accurately about 60 mg of Domperidone and transferred it to a 50 ml standard flask and sufficient methanol was added to dissolve it. Then the solution was sonicated for 10 minutes and made the volume up to the mark with methanol. Final concentration was 1200 µg /ml.

Actual weight taken,

For stock solution A (Ilaprazole) = 20.03 mg

For stock solution B (Domperidone) = 61.18 mg

Preparation of 80% Solution:

Dilute 0.8 ml of stock solution A and 0.8 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

Preparation of 90% Solution:

Dilute 0.9 ml of stock solution A and 0.9 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

Preparation of 100% Solution:

Dilute 1 ml of stock solution A and 1 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

Preparation of 110% Solution:

Dilute 1.1 ml of stock solution A and 1.1 ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

Preparation of 120% Solution:

Dilute 1.2ml of stock solution A and 1.2ml of stock solution B in 100 ml volumetric flask and make up the volume up to the mark with same diluent.

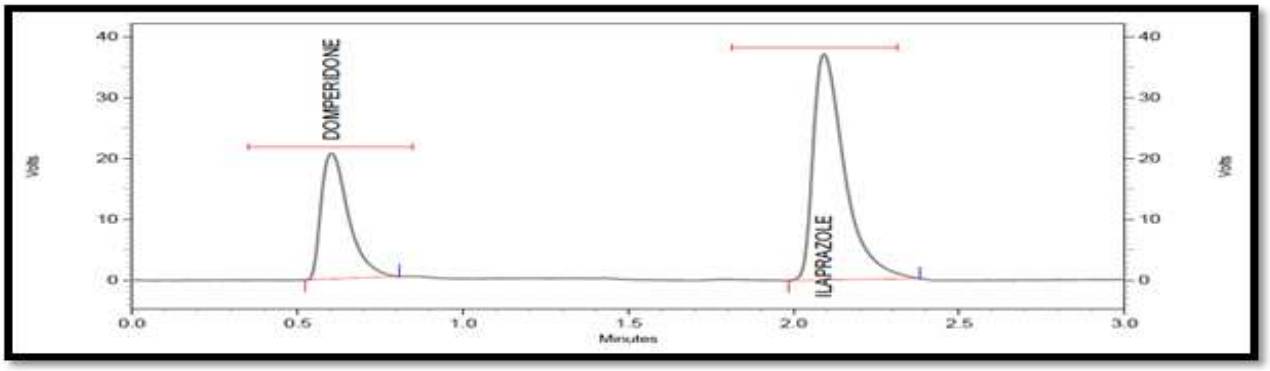


Figure 7.11: Chromatogram of linearity solution (80%)

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.603	115657	32.29	2282	0.00	1.73
IPZ	2.078	242560	67.71	2695	16.76	1.85
Total		358217	100.00			

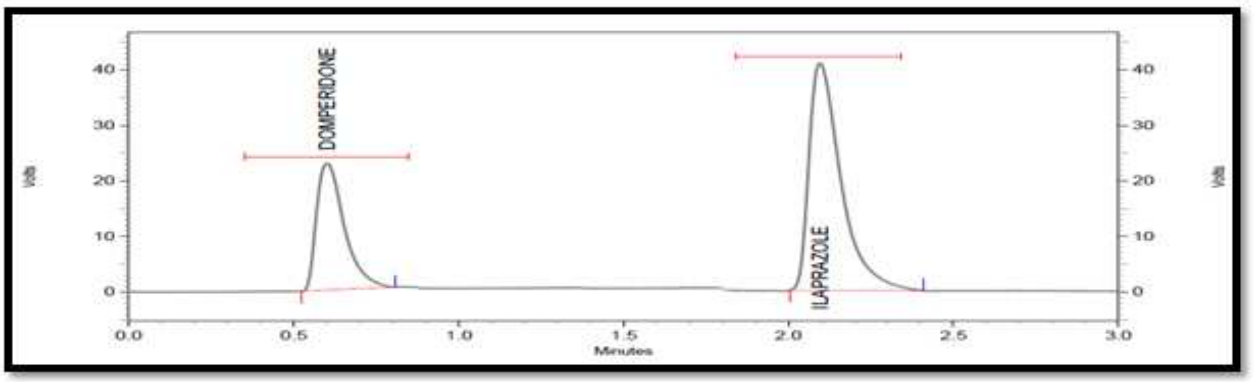


Figure 7.12: Chromatogram of linearity solution (90%)

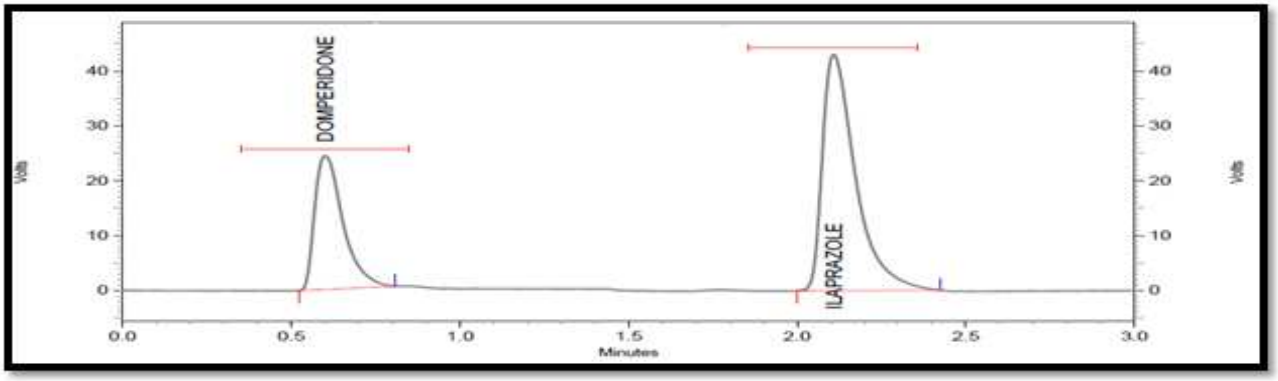


Figure 7.13: Chromatogram of linearity solution (100%)

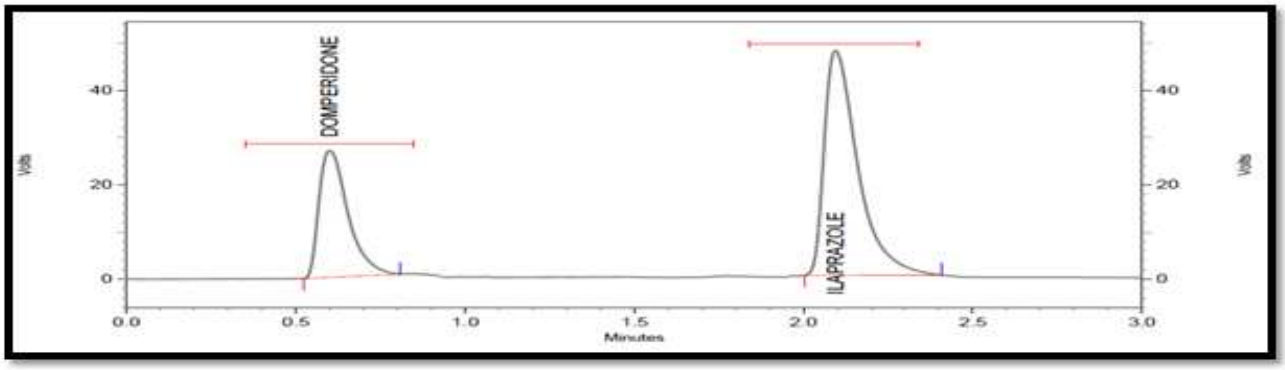


Figure 7.14: Chromatogram of linearity solution (110%)

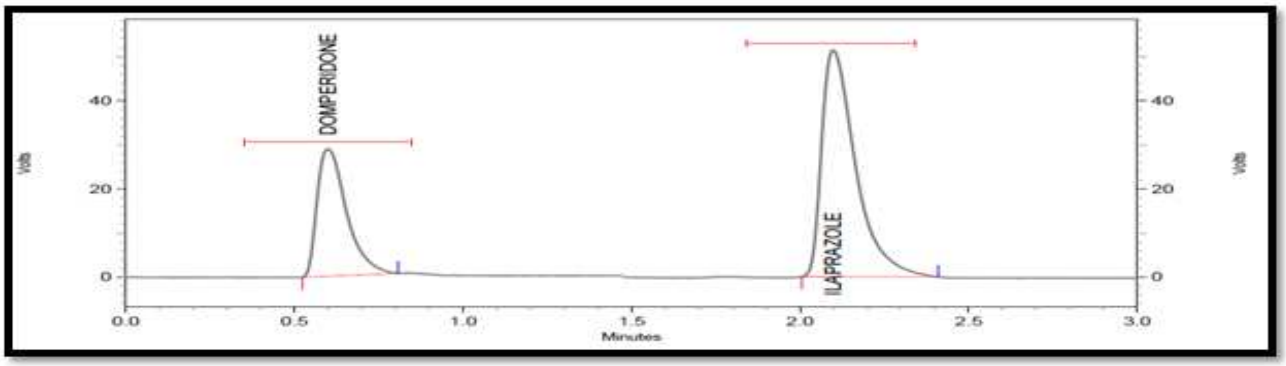


Figure 7.15: Chromatogram of linearity solution (120%)

Table 7.4: Linearity results for Domperidone and Ilaprazole

SL. NO	LINEARITY LEVEL	DOMPERIDONE (DPD)		ILAPRAZOLE (IPZ)	
		CONCENTRATION (µg/ml)	AREA	CONCENTRATION (µg/ml)	AREA
1	**80%**	9.79	115657	3.20	242560
2	**90%**	11.01	131110	3.61	275505
3	**100%**	12.24	145029	4.01	302861
4	**110%**	13.46	159996	4.41	331534
5	**120%**	14.68	174431	4.81	362008
Correlation coefficient		0.999		0.999	
Slope		11973		73371	

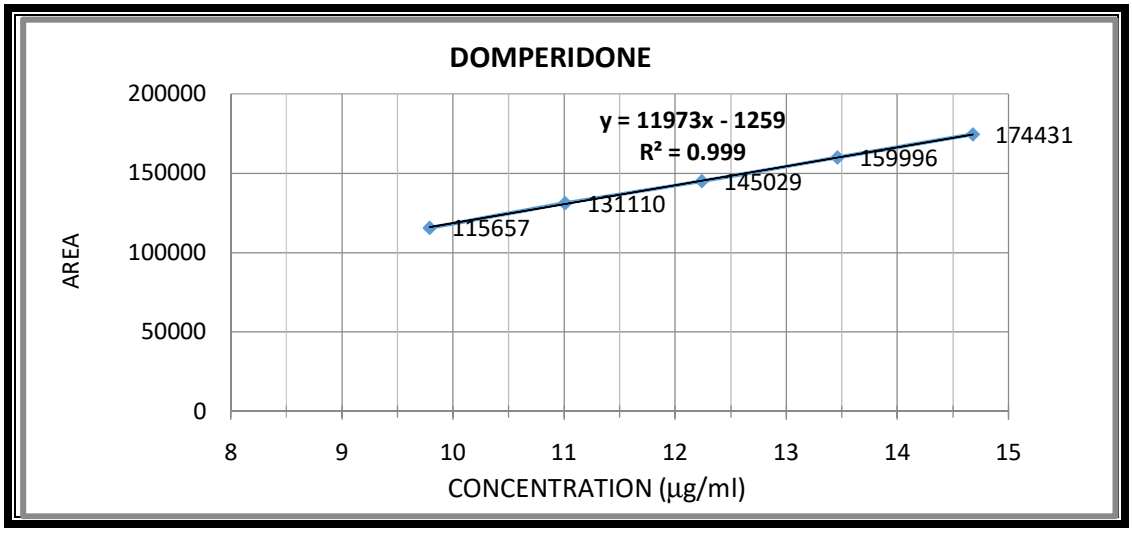


Figure 7.16: Calibration graph of Domperidone

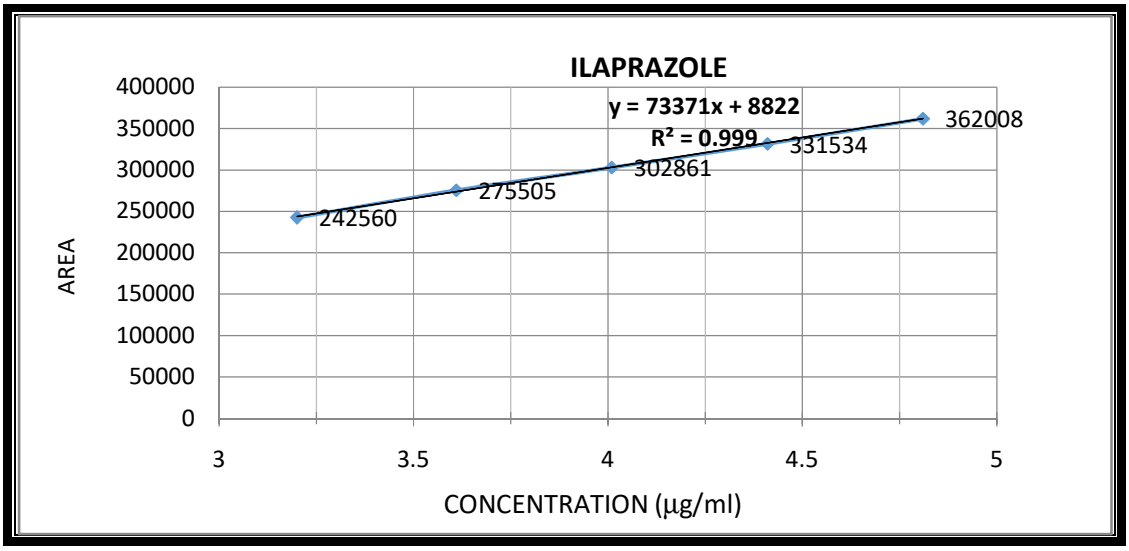


Figure 7.17: Calibration graph of Ilaprazole

Acceptance criteria: Correlation coefficient ≥ 0.997

Report: The relationship between the concentration and the peak response of Domperidone (DPD) and Ilaprazole (IPZ) were found linear in the specific range and regression coefficient for both Domperidone and Ilaprazole was found to be 0.999.

7.2.5 Accuracy

Accuracy of the method was determined by recovery experiments. Recovery study can be carried out by spiking study or swab study (usually for cleaning validation). In my experiment I performed spiking method. To the formulation, the working standards of the drug were added at the level of 100%, 110%, 120% and 130%. Prepare each level in triplicate injection and the average is taken to calculate the percentage relative standard deviation was calculated.

7.2.5.1 Procedure

Preparation of Spike Stock Solution

Weigh accurately 30 mg of Domperidone WS and 10 mg of Ilaprazole WS in a 100 ml of standard flask, dissolve in 25 ml diluent and solution was sonicated for 10 min. Make up the volume with the same diluent and filtered through 0.45 µ membrane filter.

Weight taken,

Ilaprazole = 10.01 mg

Domperidone = 30.06 mg

7.2.5.2 Preparation of 100% solution

Weighed accurately 320 mg of sample and transferred into a 25 ml volumetric flask and sufficient diluent was added to dissolve it. Then the solution was sonicated for 10 min. Volume was adjusted with the same solvent and filtered through 0.45 µ membrane filter. 1 ml of above solution was diluted with 30 ml of diluent in a 100 ml volumetric flask and final volume was adjusted with the same solvent

7.2.5.3 Preparation of 110% solution

Weighed accurately 320 mg of sample and transferred into a 25 ml volumetric flask and add sufficient diluent to dissolve it.Then the solution was sonicated for 10 min. Volume was adjusted with the same diluent and filtered through 0.45 µ membrane filter. To 1 ml of the above solution add 10 ml of standard spike solution and sufficient diluent was added to make up the volume.

7.2.5.4 Preparation of 120% solution

Weighed accurately 320 mg of sample and transferred into a 25 ml volumetric flask and add sufficient diluent to dissolve it. Then the solution was sonicated for 10 min. Volume was adjusted with the same diluent and filtered through 0.45 µ membrane filter. To 1 ml of the above solution add 20 ml of standard spike solution and sufficient diluent was added to make up the volume.

7.2.5.5 Preparation of 130% solution:

Weighed accurately 320 mg of sample and transferred into a 25 ml volumetric flask and add sufficient diluent to dissolve it. Then the solution was sonicated for 10 min. Volume was adjusted with the same diluent and filtered through 0.45 µ membrane filter. To 1 ml of the above solution add 30 ml of standard spike solution and sufficient diluent was added to make up the volume.

Table 7.5: Tabular Column for Recovery Solution

ACCURACY SOLUTION (%)	WEIGHT OF SAMPLE (mg)	THEORETICALLY PRE-EXISTING DPD (mg/capsule)	THEORETICALLY PRE-EXISTING IPZ (mg/capsule)	AMOUNT SPIKED (mg)		
				VOLUME (ml)	DPD	IPZ
100	320.01	29.99	10.00	--	--	--
110	320.03	29.99	10.00	10	0.122	0.040
120	320.03	29.99	10.00	20	0.245	0.080
130	320.02	29.99	10.00	30	0.367	0.120

$$\% \text{ Recovery} = \frac{\text{Actual calculated amount in recovery sample}}{\text{Amount spiked in to the recovery sample} + \text{Calculated theoritical pre existing amount}} \text{ X } 100$$

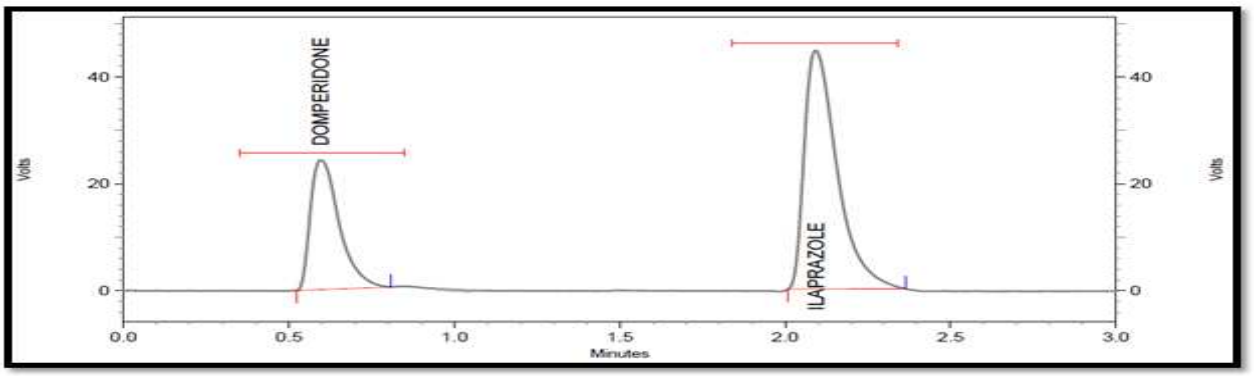


Figure 7.18: Chromatogram of accuracy 100% injection 01

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.602	147483	32.47	2269	0.00	1.76
IPZ	2.070	306783	67.53	2629	16.76	1.82
Total		454266	100.00			

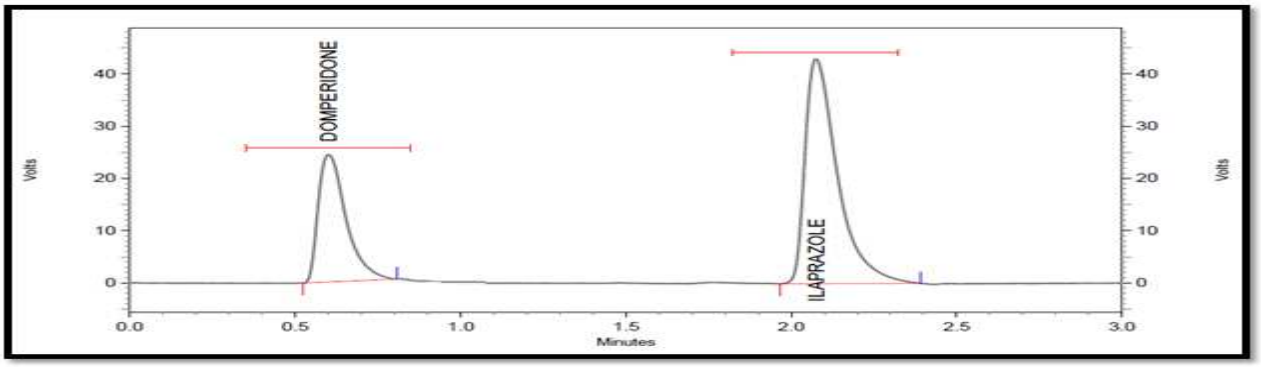


Figure 7.19: Chromatogram of accuracy 100% injection 02

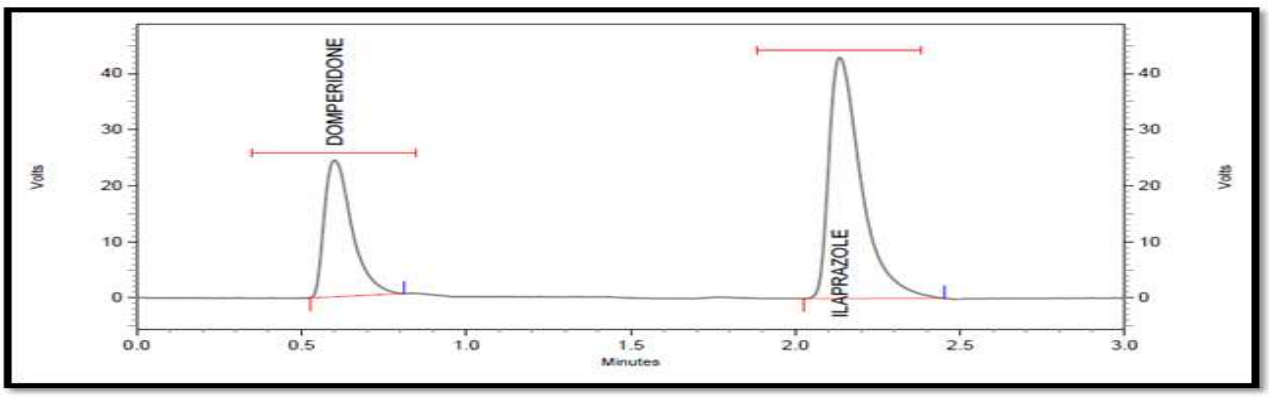


Figure 7.20: Chromatogram of accuracy 100% injection 03

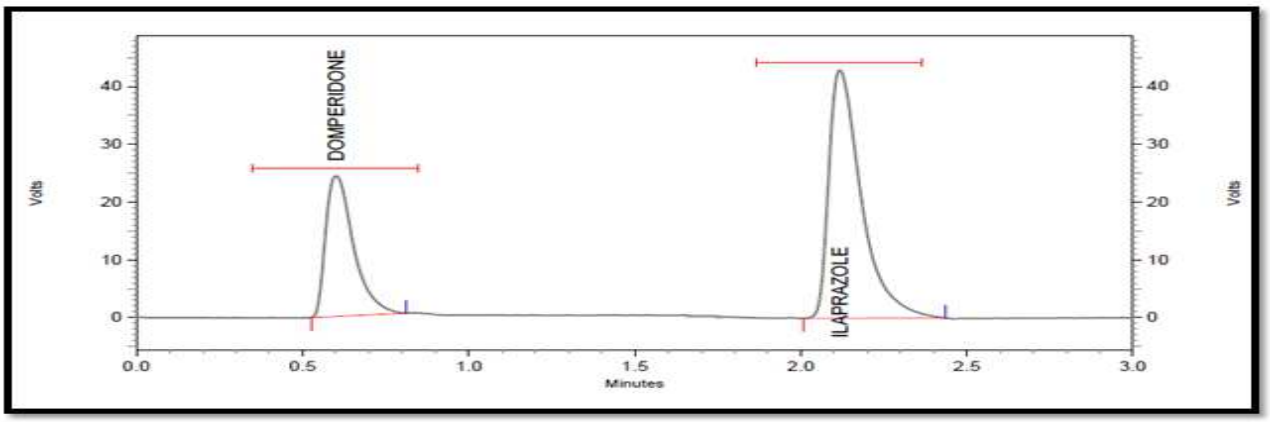


Figure 7.21: Chromatogram of accuracy 110% injection 01

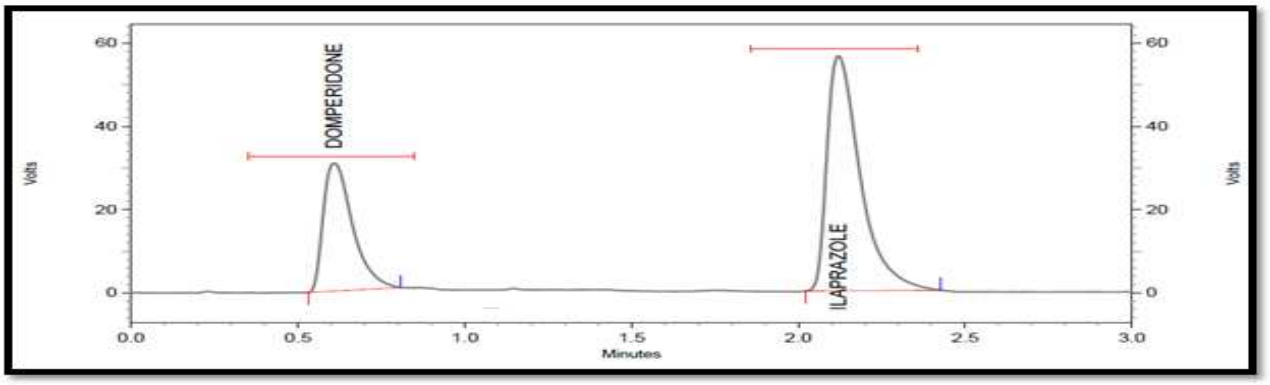


Figure 7.22: Chromatogram of accuracy 110% injection 02

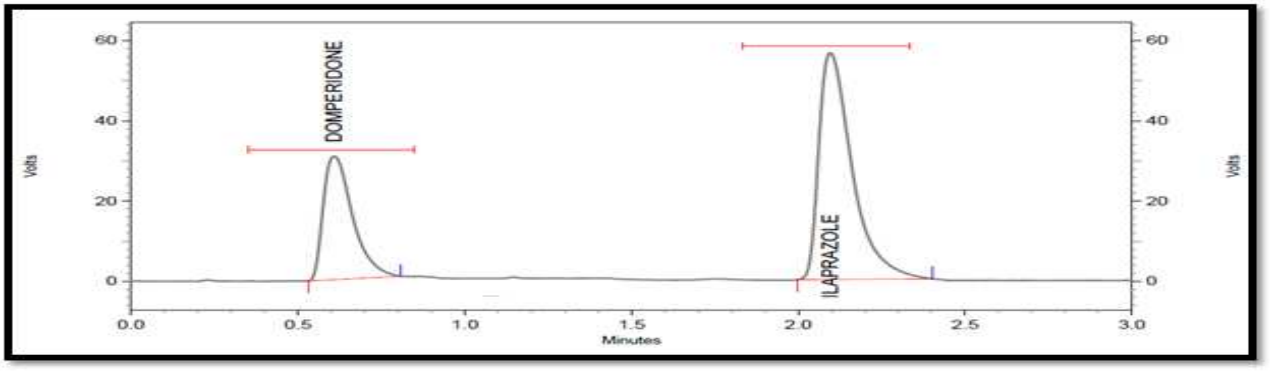


Figure 7.23: Chromatogram of accuracy 110% injection 03

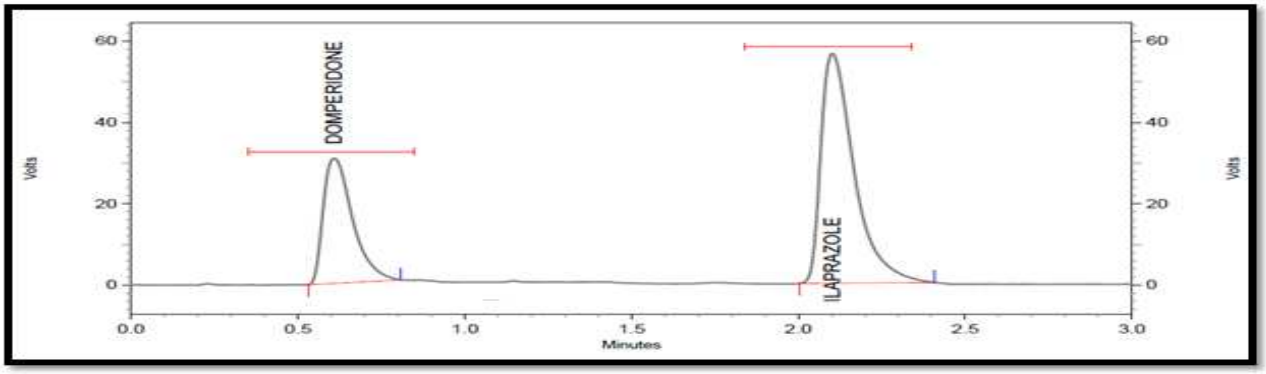


Figure 7.24: Chromatogram of accuracy 120% injection 01

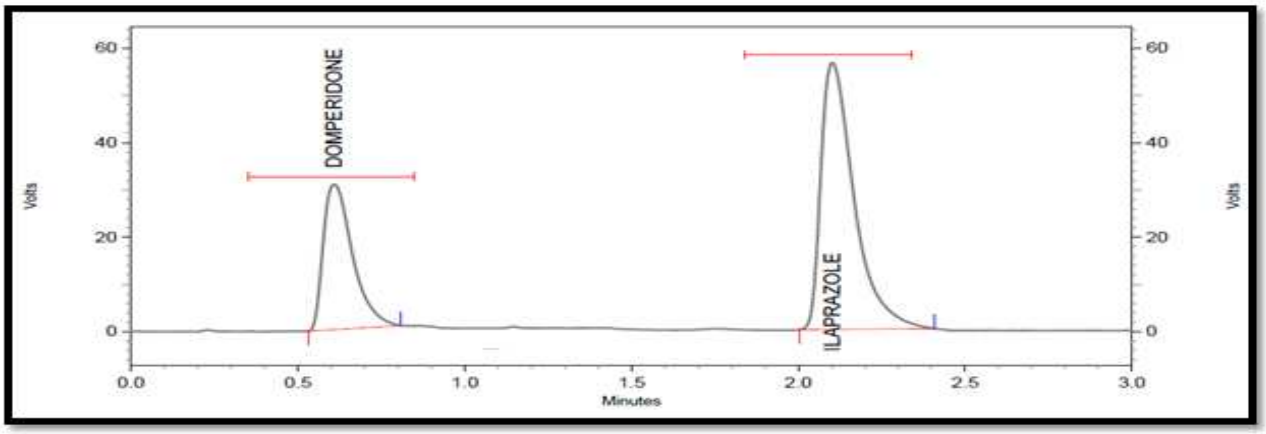


Figure 7.25: Chromatogram of accuracy 120% injection 02

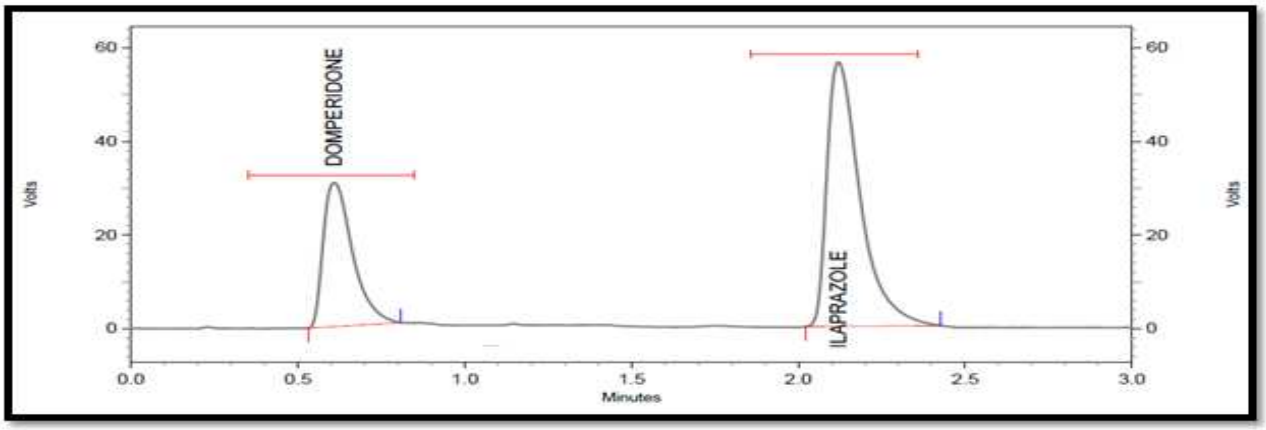


Figure 7.26: Chromatogram of accuracy 120% injection 03

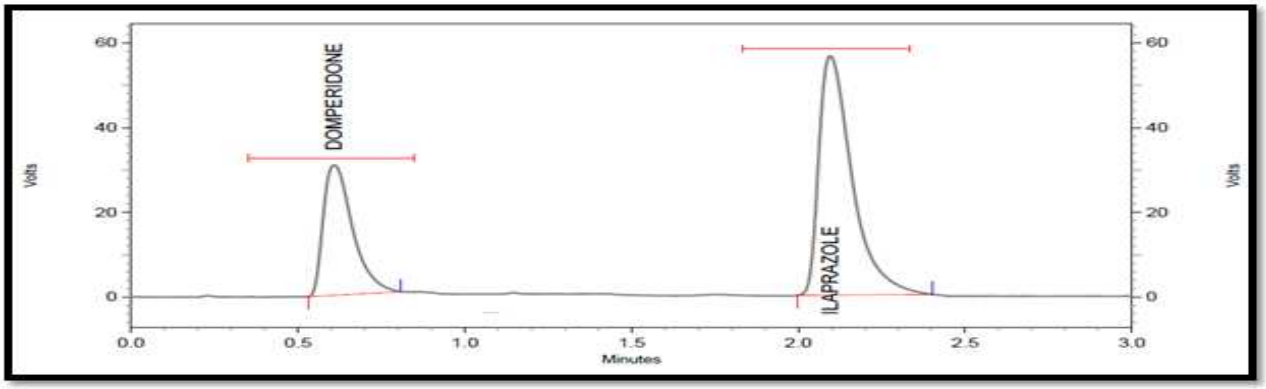


Figure 7.27: Chromatogram of accuracy 130% injection 01

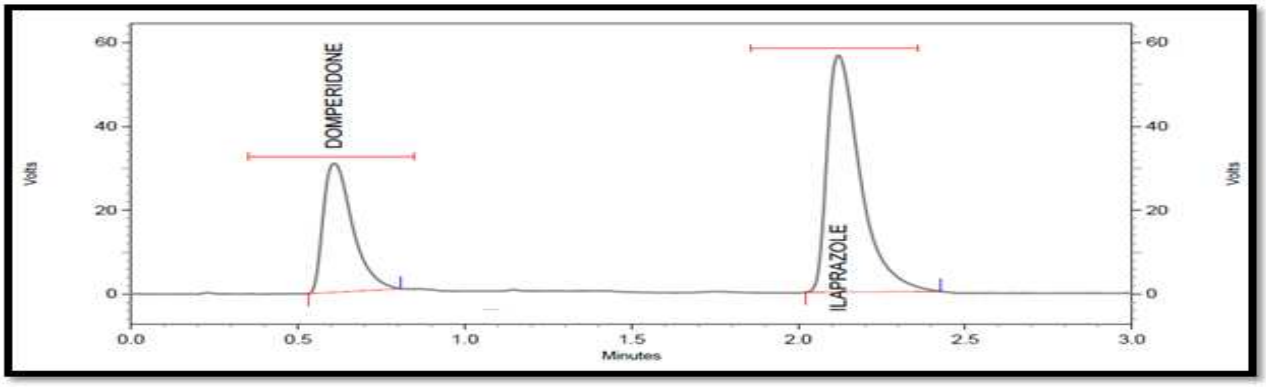


Figure 7.28: Chromatogram of accuracy 130% injection 02

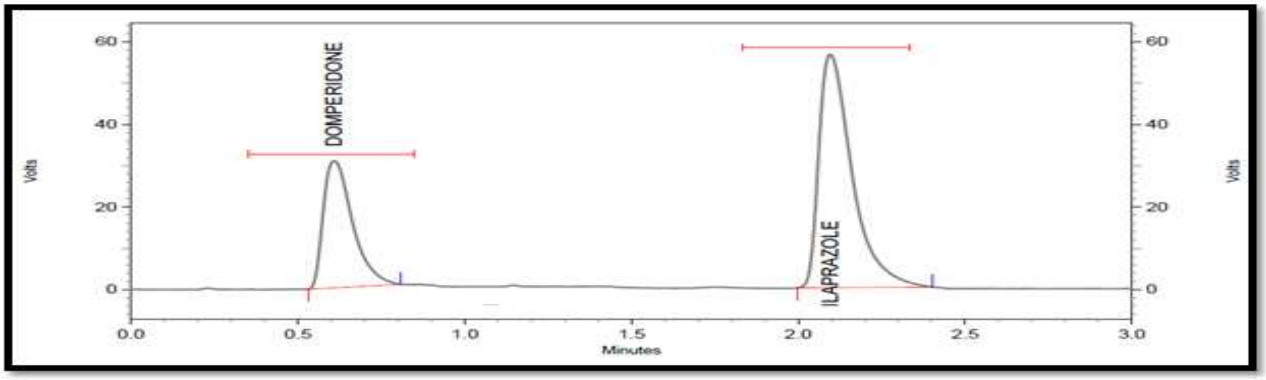


Figure 7.29: Chromatogram of accuracy 130% injection 03

Table 7.6: Accuracy results of Domperidone

% LEVEL	AREA	AMOUNT FOUND (mg)	% RECOVERY	% MEAN RECOVERY	% RSD
100%	147483	29.97	99.94	99.92	0.02
	147461	29.97	99.92		
	147437	29.96	99.90		
110%	162086	32.94	99.82	99.82	0.00
	162090	32.94	99.82		
	162083	32.94	99.82		
120%	176530	35.87	99.64	99.59	0.12
	176200	35.80	99.45		
	176601	35.89	99.68		
130%	191447	38.90	99.73	99.69	0.05
	186250	38.86	99.63		
	188412	38.90	99.71		
Average				**99.75**	**0.05**

Table 7.7: Accuracy results of Ilaprazole

% LEVEL	AREA	AMOUNT FOUND (mg)	% RECOVERY	% MEAN RECOVERY	% RSD
100%	306783	9.90	99.02	99.04	0.02
	306887	9.91	99.06		
	306890	9.91	99.06		
110%	338770	10.93	99.39	99.29	0.12
	338541	10.93	99.32		
	338005	10.91	99.17		
120%	369649	11.93	99.41	99.34	0.07
	369431	11.92	99.35		
	369117	11.91	99.26		
130%	399302	12.89	99.12	99.14	0.08
	399165	12.88	99.08		
	399771	12.90	99.23		
Average				**99.20**	**0.07**

Acceptance Criteria

The percentage recovery calculated should be between 98 to 102%.

% RSD of each level should be NMT 2.0.

Report

The average percentage recovery for DPD and IPZ was found to be 99.75% and 99.20% respectively.

The average percentage RSD for DPD and IPZ was found to be 0.05% and 0.07% respectively.

7.2.6 Precision

Demonstrate the method precision by preparing six samples as per the test method of a single batch representing the 100% of test concentration. Determine the assay of these samples and evaluate the precision of the method by the % RSD of the assay results.

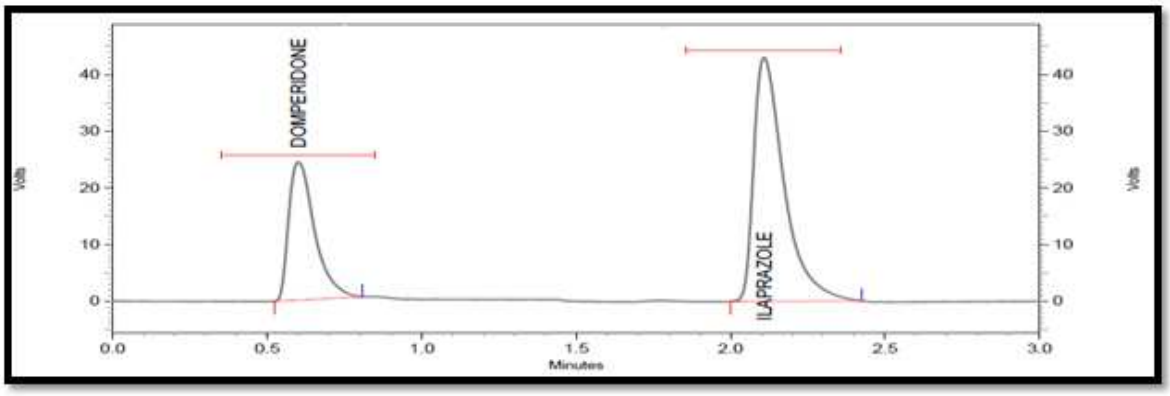


Figure 7.30: Chromatogram of Precision solution – l

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.600	147059	32.46	2295	0.00	1.67
IPZ	2.073	306022	67.54	2614	16.80	1.80
Total		453081	100.00			

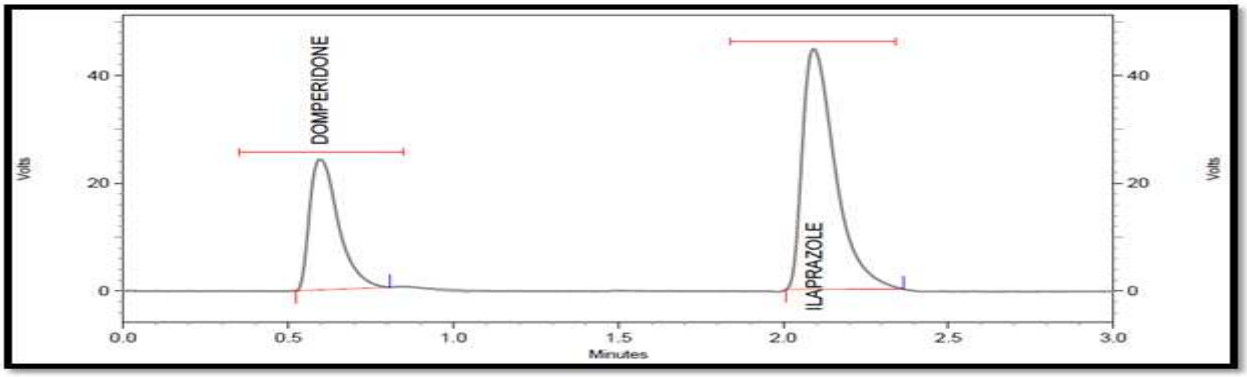


Figure 7.31: Chromatogram of Precision solution – ll

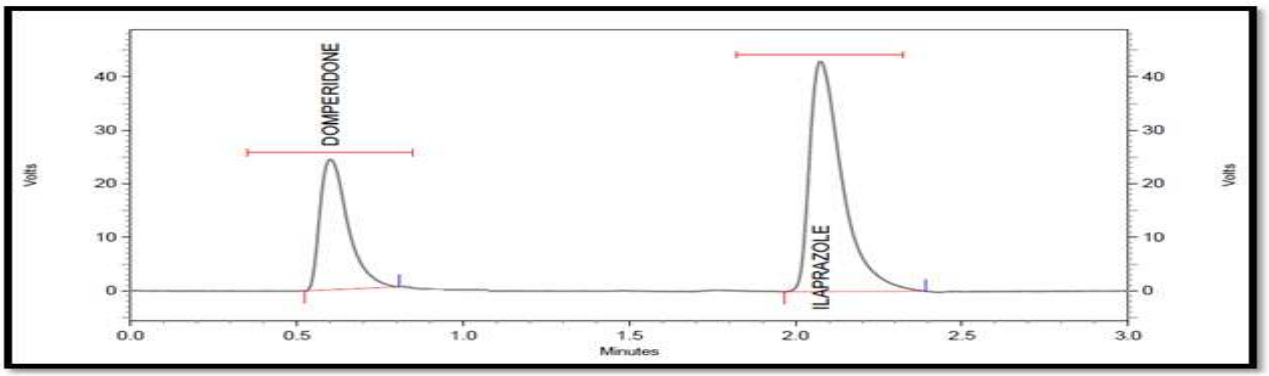


Figure 7.32: Chromatogram of Precision solution – lll

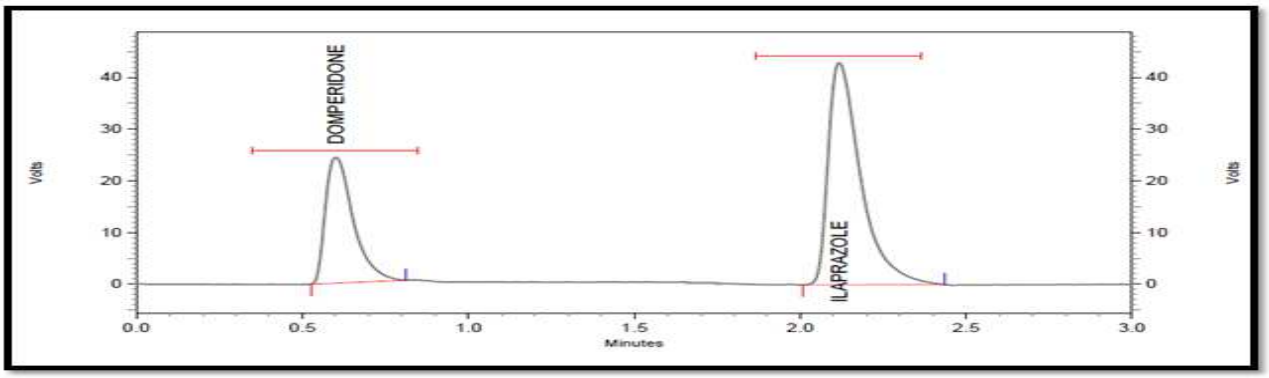


Figure 7.33: Chromatogram of Precision solution – lV

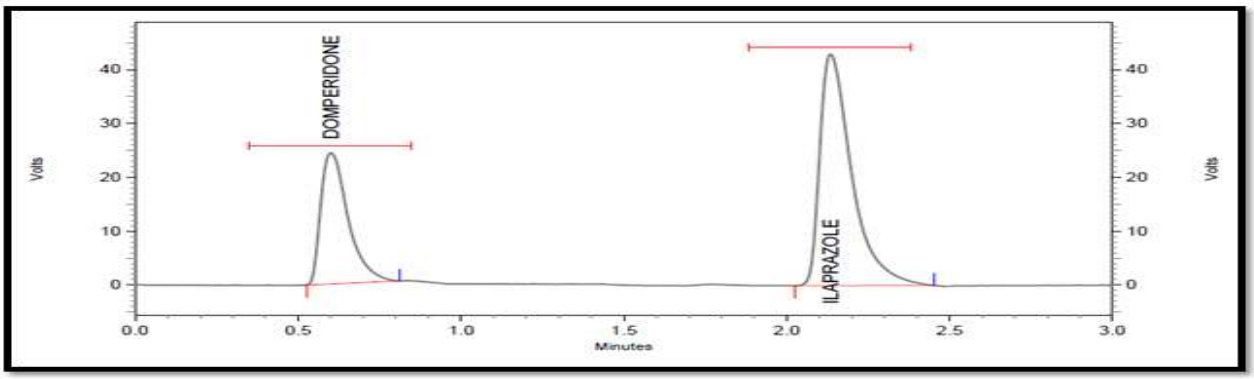


Figure 7.34: Chromatogram of Precision solution – V

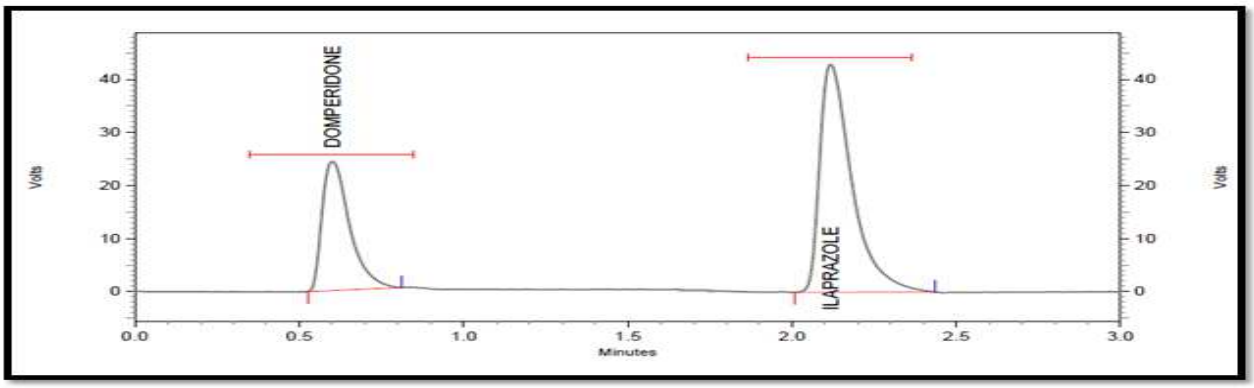


Figure 7.35: Chromatogram of Precision solution – Vl

Table 7.8: Method precision results for Domperidone & Ilaprazole

INJECTION NO.	SAMPLE WEIGHT (mg)	DOMPERIDONE (DPD)		ILAPRAZOLE (IPZ)	
		AREA	%ASSAY	AREA	%ASSAY
1	316.25	147059	100.78	306022	99.97
2	316.37	147045	100.74	306022	99.94
3	316.18	147048	100.80	306076	100.00
4	316.19	147087	100.80	306019	99.99
5	316.17	147068	100.80	306024	100.00
6	316.20	147026	100.79	306093	99.99
Average		147055.50	100.79	306042.7	99.98
Standard Deviation		20.96	0.02	32.89	0.02
% RSD		0.01	0.02	0.01	0.02

Acceptance criteria: Percentage RSD ≤ 2

Report:

- The above precision study was assessed by repeatability tests.
- The % RSD for the area and assay of DPD found to be 0.01 and 0.02 respectively.
- The % RSD for the area and assay of IPZ was found to be 0.01 and 0.2 respectively.

7.2.7 Limit of detection (LOD) & Limit of Quantitation (LOQ)

The LOD and LOQ of DPD and IPZ shall be estimated from the standard deviation of the response and the slope of the calibration curve by using the following formula

$$\text{LOD} = \frac{3.3\times\sigma}{S} \qquad \text{LOQ} = \frac{10\times\sigma}{S}$$

Where σ = the standard deviation of the response

S = the slope of the calibration curve

Table 7.9: LOD & LOQ Determination

NAME	SLOPE	STANDARD DEVIATION OF RESPONSE	LOD (µg/ml)	LOQ (µg/ml)
Domperidone (DPD)	11973	20.96	0.006	0.018
Ilaprazole (IPZ)	73371	32.89	0.001	0.004

Report:

- The LOD and LOQ of DPD for this method were found to be 0.006 µg/ml and 0.018µg/ml respectively.
- The LOD and LOQ ofIPZ for this method were found to be 0.001 µg/ml and 0.004 µg/ml respectively.

7.2.8 Robustness

Robustness of the method was checked by small deliberate changes in the method parameters such as wavelength (±2nm) and flow rate (±0.025ml) which shall not much affect in theoretical plates and peak asymmetry.

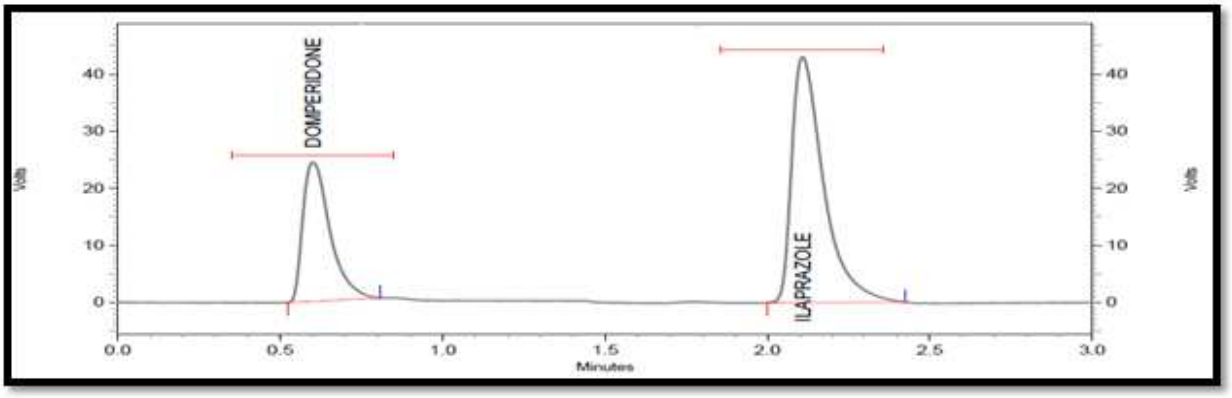


Figure 7.36: Chromatogram of robustness at wavelength 286 nm

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.602	146223	31.82	2240	0.00	1.71
IPZ	2.082	313376	68.18	2570	16.80	1.85
Total		459599	100.00			

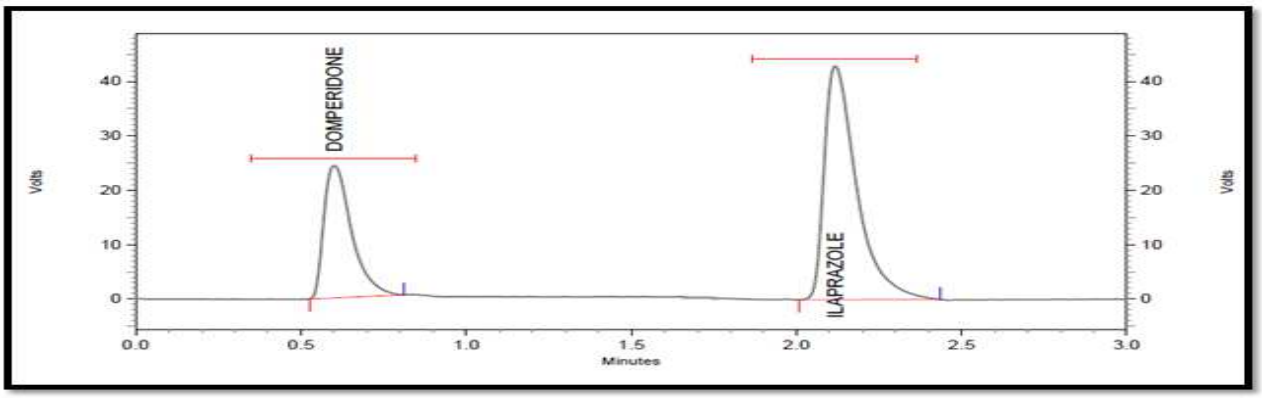


Figure 7.37: Chromatogram of robustness at wavelength 290 nm

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.602	144409	31.37	2276	0.00	1.58
IPZ	2.062	315859	68.63	2536	16.81	1.84
Total		460268	100.00			

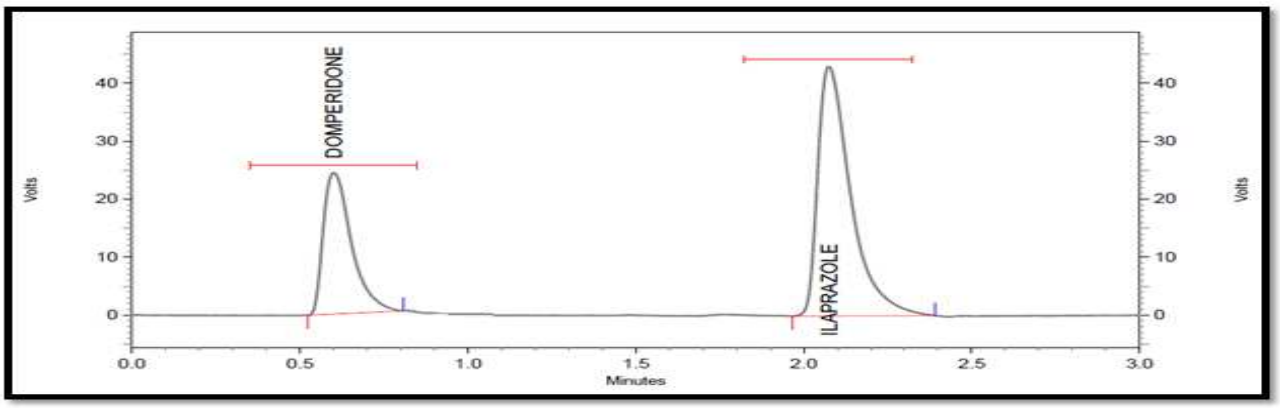


Figure 7.38: Chromatogram of robustness (flow rate at 0.375 ml /min)

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.657	146213	32.07	2228	0.00	1.59
IPZ	1.985	309729	67.93	2265	16.75	1.80
Total		455942	100.00			

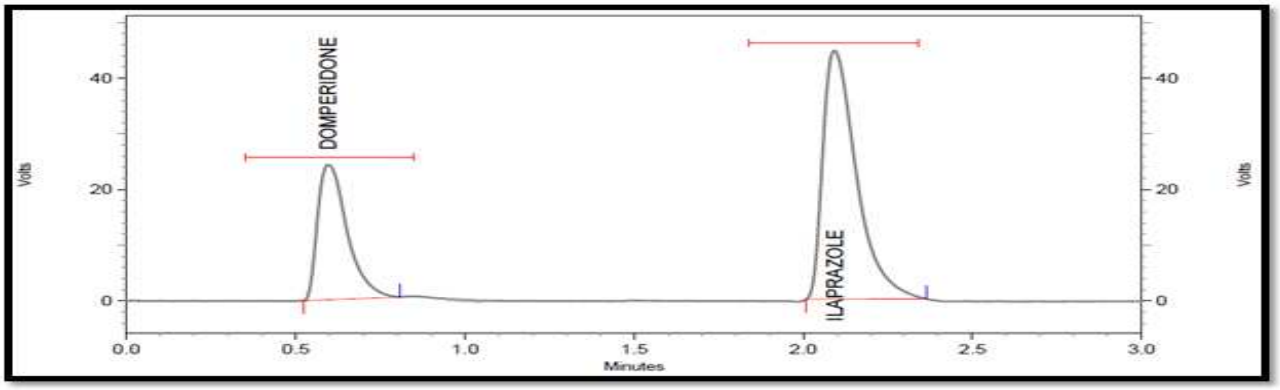


Figure 7.39: Chromatogram of robustness (flow rate at 0.425 ml /min)

DRUG	RETENTION TIME	AREA	AREA %	THEORETICAL PLATES (USP)	RESOLUTION (USP)	ASYMMETRY
DPD	0.552	143529	32.13	2254	0.00	1.64
IPZ	2.097	303132	67.87	2598	16.77	1.94
Total		446661	100.00			

Table 7.10: Robustness observation of Domperidone

SL. NO	PARAMETER	CONDITION	RETENTION TIME (min)	AREA	THEORETICAL PLATES (USP)	ASYMMETRY
1	Wavelength variation	286 nm	0.602	146223	2240	1.71
		288 nm	0.598	145852	2249	1.7
		290 nm	0.602	144409	2276	1.58
% RSD			--	0.66	--	--
2	Flow Rate	0.375ml /min	0.657	146213	2228	1.59
		0.400ml /min	0.598	145852	2249	1.7
		0.425ml /min	0.552	143529	2254	1.64
% RSD			--	1.00	--	--

Table 7.11: Robustness observation of Ilaprazole

SL. NO	PARAMETER	CONDITION	RETENTION TIME (min)	AREA	THEORETICAL PLATES (USP)	ASYMMETRY
1	Wavelength variation	286 nm	2.082	313376	2570	1.85
		288 nm	2.072	306090	2589	1.85
		290 nm	2.062	315859	2536	1.84
% RSD			--	1.63	--	--
2	Flow Rate	0.375ml /min	1.985	309729	2265	1.8
		0.400ml /min	2.072	306090	2589	1.85
		0.425ml /min	2.097	303132	2598	1.94
% RSD			--	1.08	--	--

Acceptance Criteria: % RSD ≤ 2

Theoretical plates (USP) ≥ 2000

Asymmetry ≤ 2

Report:

The robustness was tested by changing the wavelength and flow rate in the chromatographic parameters. It has been found that change in flow rate leads to little change in retention time.

The percentage RSD for wavelength variation of Domperidone and Ilaprazole was found to 0.66% and 1.63% respectively.

The percentage RSD for flow rate variation of Domperidone and Ilaprazole was found to 1.00% and 1.08% respectively.

RESULTS AND DISCUSSION

8. RESULTS AND DISCUSSION

8.1 RESULTS

The analytical method for the Domperidone and Ilaprazole by UPLC was established then optimized and applied on pharmaceutical dosage forms.

Various trials were performed in order to optimize the following analytical parameter like choice of column, mobile phase composition, flow rate and injection volume and the results are shown in table 8.1.

Table 8.1: Tabular column for analytical parameters

Column	C18(50 X 2.1 mm, 1.9 µm); Make Thermo
Mobile phase composition	Water : Methanol : ACN : Acetic Acid (30:20:50:0.3) finally adjust the pH to 5.0 with Triethylamine
Diluent	Methanol
Flow rate	0.4 ml/min
Column temperature	Ambient
Injection volume	10 µl
Pump mode	Isocratic
PDA detection	288 nm

As per above mentioned analytical method the assay of Domperidone and Ilaprazole for marketed formulation (Capsule dosage form) was carried out and the content and percentage purity are mentioned in table 8.2.

Table 8.2: Results of assay of DPD&IPZ in capsule dosage form

SL. NO	DRUG NAME	LABEL CLAIM	CONTENT PRESENT	PERCENTAGE PURITY
1	Domperidone	30 mg	29.99 mg	99.98%
2	Ilaprazole	10 mg	10.00 mg	100.00%

Table 8.3:Results of Validation Parameters

Validation Parameter	Acceptance Criteria	Observation		Remarks
		DPD	IPZ	
Specificity	The peaks of diluent and excipients should not interfere with the main peak and peak of standard and sample should be identical with near retention time.	Complies	Complies	-
System Suitability	Asymmetry NMT 2.0	1.69	1.82	Complies
	Theoretical plates NMT 2000	2264.80	2620	
	%RSD of area NMT 2.0	0.381	0.388	
Linearity	Correlation Coefficient NLT 0.997	0.999	0.999	Complies
Accuracy	The % recovery at each spike level shall be NLT 98.0% and NMT 102.0% of the added amount.	99.59-99.92%	99.04-99.34%	Complies
	% RSD of each level should be NMT 2.0	Average % RSD = 0.05	Average % RSD = 0.07	
Precision	The % RSD of area for the six determinations shall be NMT 2.0.	0.01	0.01	Complies
	The % RSD of assay for the six determinations shall be NMT 2.0.	0.02	0.02	
LOD (µg/ml)	Not specified	0.006	0.001	Suitable
LOQ (µg/ml)	Not specified	0.018	0.004	Suitable
Robustness	Theoretical plates (USP) ≥ 2000	Complies	Complies	-
	Asymmetry ≤ 2	Complies	Complies	

8.2 DISCUSSION

A simple and rapid RP-UPLC method was developed for the simultaneous estimation of Domperidone and Ilaprazole in pharmaceutical dosage form. For the analysis, the mobile phase selected consists of mixture of **Water : Methanol : ACN : Acetic Acid (30:20:50:0.3)**and finally **pH** was adjusted to **5.0** with **Triethylamine**. Column suitable for this method was found C18 (50 X 2.1 mm, 1.9 µm); Make Thermo.

From the spectral characteristic, the detection was carried out by using PDA detector at **288 nm**. The retention time is for DPD&IPZ was found **0.598 min**&**2.072 min** respectively. The asymmetry factor was found to be **1.70** and **1.85**, which indicates symmetrical nature of the peak. Resolution (USP) between two peaks was **16.81** which will satisfy the acceptance criteria.

The specificity was performed with respect blank and identification and the chromatogram showed was identical with near retention time with standard.

The System suitability parameter such as retention time, number of theoretical plates, and asymmetry factor, % RSD of area were recorded and found to be within limits mentioned in table 7.1 & 7.2.

With the optimized conditions, linearity range was fixed as (80-120) % i.e. (80-120) µg/ml of DPD and (8-12) µg/ml of IPZ. Linearity range was evaluated by the visual inspection of plot of peak area as a function of analyte concentration and the corresponding calibration graphs were shown in figure 6.15 & 6.16and results are shown in table 6.3. From the linearity studies, the specified concentration range was determined. It was observed that Domperidone and Ilaprazole were linear in the range of (9.79-14.68) µg/ml and (3.20-4.81) µg/ml respectively for the target concentrations. The regression equation of Domperidone and Ilaprazole for concentration over its

peak area ratio was found to be **y=11973x-1259(R^2 = 0.999)** and **y=73371x+8822(R^2 = 0.999)** where y is the peak area ratio and x is the concentration of DPD&IPZ in µg/ml.

The validation of proposed method was verified by recovery (spiking) studies. The percentage recovery range was found between **99.59-99.92%** for DPD and **99.04-99.34%** for IPZ. This is a good index of accuracy, specificity and repeatability of the method. The results were tabulated in table 7.6 & 7.7. All parameters including flow rate, temperature, detection, wavelength and sensitivity are maintained constant throughout the procedure.

The validation of the proposed method was verified by method precision where repeatability study was performed by preparing six samples as per the test method of a single batch representing the 100% of test concentration. The %RSD of assay and area for method precision were calculated and the data was tabulated and shown in table 7.8.

LOD and LOQ were calculated from the linearity parameter data. The results were tabulated and shown in table 7.9.

Robustness studies were made by varying wavelength (±2 nm) and flow rate (±0.025 ml/min) and the results were mentioned in table 7.10 & 7.11.

SUMMARY AND CONCLUSION

8. SUMMARY & CONCLUSION

8.1 SUMMARY

From the literature review it was found that no UPLC method was carried out for the simultaneous determination of Domperidone and Ilaprazole in capsule dosage form. Various trials were conducted by varying the wavelength, column, mobile phase, and flow rate and injection volume and the parameters for UPLC method for the determination of DPD and IPZcapsule dosage form were optimized.

The scope of the present work is to expand the optimization of the chromatographic conditions and to develop new RP-UPLC method. A series of mobile phases were tried and among the various mobile phase comprising of mixture of **Water : Methanol : ACN : Acetic Acid (30:20:50:0.3) (pH 5.0, adjusted with Triethylamine)** was chosen as an ideal mobile phase, since it a good resolution and peak shapes with perfect optimization. The detection was carried out at **288 nm** by using PDA detector. The flow rate was optimized at 0.4 ml/min.

The Specificity of DPD and IPZ were shown in Chromatograms. There was no interference in this method and good separation between all peaks. It means no impurity was interfered and also reveals that commonly used excipients and additives present in the capsule dosage form were not interfering in the proposed methods. It was also found that the retention time of sample DPD and IPZ were identical corresponding to standard DPD and IPZ.

The system suitability was found within the limits. The % RSD for DPD and IPZ were found **0.381% and 0.388%** respectively. Theoretical plates, resolution and Asymmetry factor also found suitable.

The linearity obtained from calibration graph shows that DPD and IPZ were linear in the range of (9.79-14.68) µg/ml and (3.20-4.81) µg/ml respectively. Correlation coefficient in the range of (9.79-14.68)µg/ml for DPD and (3.20-4.81) µg/ml for IPZ were calculated and found 0.999 and 0.999 respectively.

In assay of marketed formulation the percentage purity of DPD and IPZ were found 102.53 % and 100.00% respectively which indicate that the method can be used to determine the percentage purity of Domperidone and Ilaprazole in capsule dosage form.

Accuracy study was carried out by spiking method where calculated known amount of standard were added for different Percentage levels (100% - 130%). The average percentage recovery for DPD and IPZ was found to be 99.75% and 99.20% respectively. The average percentage RSD for DPD and IPZ was found to be 0.05% and 0.07% respectively.

The limits of % recovery studies are in the range of 98-102% and the results found that recovery values of pure drugs from the solution were as well, which indicates that the method is accurate.

Method Precision was assessed by repeatability tests (6 determinations at the level of 100% concentration). The % RSD for the area and assay of DPD found to be 0.01 and 0.02 respectively. The % RSD for the area and assay of IPZ was found to be 0.01 and 0.2 respectively.

Hence method is precise for DPD and IPZ. The complete data regarding the precision was shown in table11.

Based on standard deviation response (standard deviation of y intercept obtained from the calibration curve) and slope, the Limit of detection (LOD) and Limit of Quantitation (LOQ) were calculated for DPD and IPZ. The Limit of detection (LOD) of DPD and IPZ were found 0.006µg/ml

and 0.001µg/ml respectively. And the Limit of Quantitation (LOQ) of DPD and IPZ were found 0.018µg/ml and 0.004µg/ml respectively.

Robustness of the method was checked by small deliberate changes in the method parameters such as wavelength (±2nm) and flow rate (±0.2ml) but these changes did not affect the chromatographic parameter and the method results which indicate that the method is robust.

8.2 CONCLUSION

Thus the proposed method was found to be simple, accurate, precise and rapid for simultaneous estimation of Domperidone and Ilaprazole in pharmaceutical capsule dosage form and could be used for routine analysis. All the parameters meet the criteria of ICH guidelines for method validation and found to be simple, sensitive, accurate and precise. It can therefore be concluded that the reported method is more economical and can find practical application for simultaneous analysis of the Domperidone (DPD) and Ilaprazole (IPZ) in their combined dosage forms both in research and quality control laboratories

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